DIAGNOSTIC ROLE OF PROCALCITONIN IN NEONATAL SEPSIS IN TERTIARY CARE HOSPITAL

DISSERTATION SUBMITTED TO

In partial fulfillment of the requirement for the degree of

DOCTOR OF MEDICINE IN MICROBIOLOGY

(Branch IV) M. D. (MICROBIOLOGY)

of

THE TAMIL NADU DR. M. G. R MEDICAL UNIVERSITY

CHENNAI- 600032

DEPARTMENT OF MICROBIOLOGY

TIRUNELVELI MEDICAL COLLEGE

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BONAFIDE CERTIFICATE

This is to certify that the dissertation entitled **“Diagnostic role of Procalcitonin in neonatal sepsis in tertiary care hospital”** submitted by **Dr.D.Jeyaganguli** to the Tamilnadu Dr. M.G.R Medical University, Chennai, in partial fulfillment of the requirement for the award of M.D. Degree Branch – IV (Microbiology) is a bonafide research work carried out by her under direct supervision & guidance.

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This is to certify that the Dissertation **“Diagnostic role of procalcitonin in neonatal sepsis in tertiary care hospital”** presented here in by **Dr.D.Jeyaganguli** is an original work done in the Department of Microbiology, Tirunelveli Medical College Hospital,Tirunelveli for the award of Degree of M.D. (Branch IV) Microbiology under my guidance and supervision during the academic period of 2015 -2018.

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I solemnly declare that the dissertation titled**"Diagnostic role of procalcitonin in neonatal sepsis in tertiary care hospital"** is done by me at Tirunelveli Medical College hospital, Tirunelveli. I also declare that this bonafide work or a part of this work was not submitted by me or any others for any award, degree, or diploma to any other University, Board, either in or abroad.

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Place: Tirunelveli
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ACKNOWLEDGEMENT

I am grateful to the **Dean, Dr. Siddhi athiya munaivara M.D.,** Tirunelveli Medical College, Tirunelveli for all the facilities provided for the study.

I take this opportunity to express my profound gratitude to **Dr.C.Revathy,M.D.,** Professor and Head, Department of Microbiology, Tirunelveli Medical College, whose kindness, guidance and constant encouragement enabled me to complete this study.

I wish to thank **Dr. V.Ramesh Babu, M.D**., Professor ,Department of Microbiology, Tirunelveli Medical College, for his valuable guidance for the study.

I am deeply indebted to **Dr. S. Poongodi@ Lakshmi,M.D.,** Professor, Department of Microbiology, Tirunelveli Medical College, who helped me offering most helpful suggestions and corrective comments.

I am very grateful to **Dr.B.Sorna jeyanthi,M.D.,** Professor, Department of Microbiology, Tirunelveli Medical College, for the constant support rendered throughout the period of study and encouragement in every stage of this work.

I am highly obliged to Senior Assistant Professors **Dr.B.Cinthujah,M.D., Dr. G.Velvizhi, M.D., Dr. G.Sucila Thangam, M.D, Dr V.P.Amudha M.D.**and Assistant Professors **Dr I.M Regitha M.D., Dr.Gowri, M.D ,Dr.Kanagapriya,M.D. ,** Department of Microbiology, Tirunelveli Medical College, for their evincing keen interest, encouragement, and corrective comments during the research period.

Special thanks are due to my co-postgraduate colleagues **Dr. S. Punitha ranjitham, Dr.R.P.R.Suyambu Meenakshi, Dr.V.Uma Maheswari** and **Dr.Ambuja Sekhar** for never hesitating to lend a helping hand throughout the study.

I would also wish to thank my junior post-graduate colleagues, **Dr.M.SaiShruti , Dr.E.Manimala, Dr. Maya Kumar, Dr. L.Gracia Paul and Dr.R.Uma Maheswari** for their help and support.

Thanks are due to the, Messer V.Parthasarathy, V.Chandran, S.Pannerselvam, S.Santhi, S.Venkateshwari, S.Arifal Beevi, S.Abul Kalam, A.Kavitha, ,T.Jeya, K.Sindhu, Mangai,Manivannan, K.Umayavel, Sreelakshmi and other supporting staffs for their services Rendered.

I am indebted to my husband, my parents and my family for not only their moral support but also for tolerating my dereliction of duty during the period of my study.

And of course, I thank the Almighty for His presence throughout my work. Without the Grace of God nothing would have been possible.

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PROTOCOL TITLE: DIAGNOSTIC ROLE OF PROCALCITONIN IN NEONATAL SEPSIS IN TERTIARY CARE HOSPITAL.

PRINCIPAL INVESTIGATOR: Dr.D. JEYAGANGULI, MBBS.,

DESIGNATION OF PRINCIPAL INVESTIGATOR: POST GRADUATE IN MICROBIOLOGY

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Dear , Dr. D. Jeyaganguli, MBBS., The Tirunelveli Medical College Institutional Ethics Committee (TIREC) reviewed and discussed your application during the IEC meeting held on 05.08.2016.

THE FOLLOWING DOCUMENTS WERE REVIEWED AND APPROVED

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2.	Study Protocol	☐
3.	Department Research Committee Approval	☐
4.	Patient Information Document and Consent Form in English and Vernacular Language	☐
5.	Investigator's Brochure	☐
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10.	Investigator's Undertaking	☐
11.	DCGI/DGFT approval	☐
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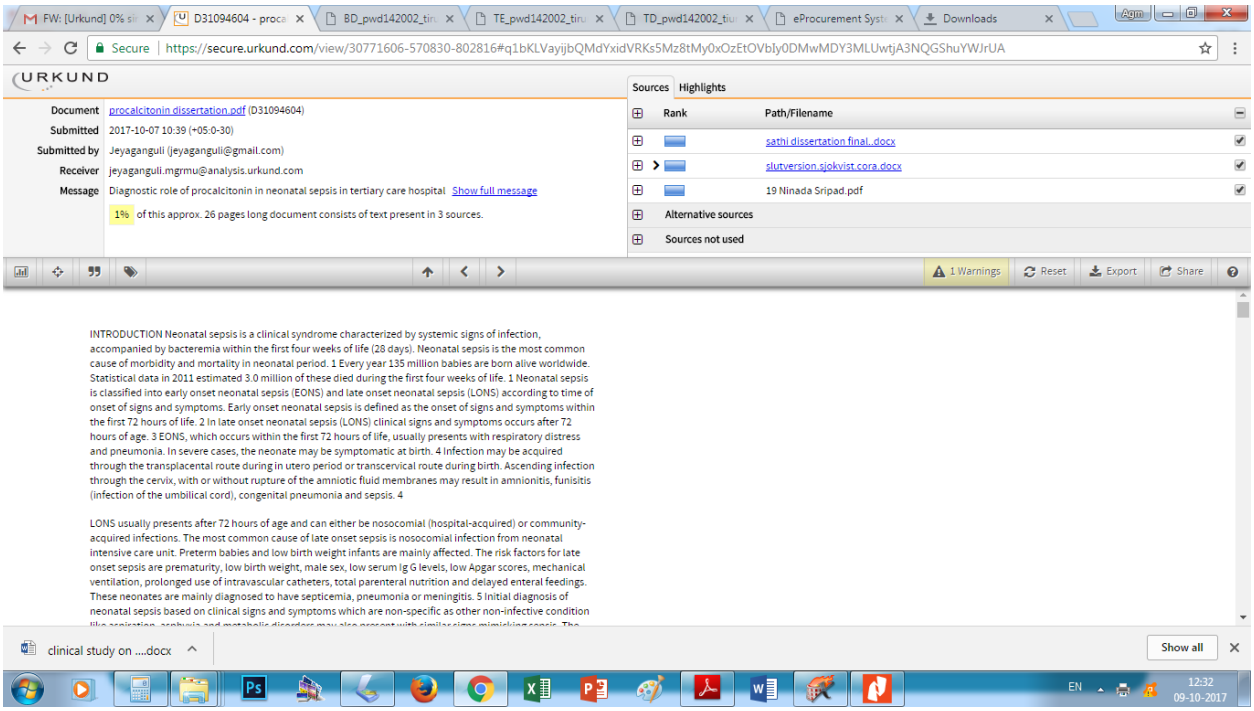
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INTRODUCTION Neonatal sepsis is a clinical syndrome characterized by systemic signs of infection, accompanied by bacteremia within the first four weeks of life (28 days). Neonatal sepsis is the most common cause of morbidity and mortality in neonatal period. 1 Every year 135 million babies are born alive worldwide. Statistical data in 2011 estimated 3.0 million of these died during the first four weeks of life. 1 Neonatal sepsis is classified into early onset neonatal sepsis (EONS) and late onset neonatal sepsis (LONS) according to time of onset of signs and symptoms. Early onset neonatal sepsis is defined as the onset of signs and symptoms within the first 72 hours of life. 2 In late onset neonatal sepsis (LONS) clinical signs and symptoms occurs after 72 hours of age. 3 EONS, which occurs within the first 72 hours of life, usually presents with respiratory distress and pneumonia. In severe cases, the neonate may be symptomatic at birth. 4 Infection may be acquired through the transplacental route during in utero period or transcervical route during birth. Ascending infection through the cervix, with or without rupture of the amniotic fluid membranes may result in amnionitis, funisitis (infection of the umbilical cord), congenital pneumonia and sepsis. 4
LONS usually presents after 72 hours of age and can either be nosocomial (hospital-acquired) or community-acquired infections. The most common cause of late onset sepsis is nosocomial infection from neonatal intensive care unit. Preterm babies and low birth weight infants are mainly affected. The risk factors for late onset sepsis are prematurity, low birth weight, male sex, low serum Ig G levels, low Apgar scores, mechanical ventilation, prolonged use of intravascular catheters, total parenteral nutrition and delayed enteral feedings. These neonates are mainly diagnosed to have septicemia, pneumonia or meningitis. 5 Initial diagnosis of neonatal sepsis based on clinical signs and symptoms which are non-specific as other non-infective condition
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ABBREVIATIONS

ADCC – Antibody depended cell mediated cytotoxicity

ANC – Absolute neutrophil count

CRP – C-reactive protein

ELISA – Enzyme linked immuosorbent assay

EONS – Early onset neonatal sepsis

ESBL – Extended spectrum beta lactamase

ESR - Erythrocyte sedimentation rate

IL – Interleukin

LONS – Late onset neonatal sepsis

LBW – Low birth weight

MRSA – Methicillin resistant Staphylococcus aureus

NBW – Normal birth weight

PCT – Procalcitonin

TNF-α – Tumour necrosis factor α

CONTENTS

1. INTRODUCTION

Neonatal sepsis is a clinical syndrome characterized by systemic signs of infection, accompanied by bacteremia within the first four weeks of life (28 days). Neonatal sepsis is the most common cause of morbidity and mortality in neonatal period.[1]Every year 135 million babies are born alive worldwide. Statistical data in 2011 estimated 3.0 million of these died during the first four weeks of life.[1]

Neonatal sepsis is classified into early onset neonatal sepsis (EONS) and late onset neonatal sepsis (LONS) according to time of onset of signs and symptoms. Early onset neonatal sepsis is defined as the onset of signs and symptoms within the first 72 hours of life.[2]In late onset neonatal sepsis (LONS) clinical signs and symptoms occurs after 72 hours of age.[3]

EONS, which occurs within the first 72 hours of life, usually presents with respiratory distress and pneumonia. In severe cases, the neonate may be symptomatic at birth.[4]Infection may be acquired through the transplacental route during in utero period or transcervical route during birth. Ascending infection through the cervix, with or without rupture of the amniotic fluid membranes may result in amnionitis, funisitis (infection of the umbilical cord), congenital pneumonia and sepsis.[4]

LONS usually presents after 72 hours of age and can either be nosocomial (hospital-acquired) or community-acquired infections. The most common cause of late onset sepsis is nosocomial infection from neonatal intensive care unit. Preterm babies and low birth weight infants are mainly affected. The risk factors for late onset sepsis are prematurity, low birth weight, male sex, low serum Ig G levels, low Apgar scores, mechanical ventilation, prolonged use of intravascular catheters, total parenteral nutrition and delayed enteral feedings. These neonates are mainly diagnosed to have septicemia, pneumonia or meningitis.[5]

Initial diagnosis of neonatal sepsis based on clinical signs and symptoms which are non-specific as other non-infective condition like aspiration, asphyxia and metabolic disorders may also present with similar signs mimicking sepsis. The problem of symptom wise false positivity in diagnosing sepsis resulting in unwarranted initiation of empirical antibiotic therapy may lead to development of drug resistance, prolonged hospital stay, increased treatment cost and the separation of the neonates from their mothers.

Diagnosis of neonatal sepsis is broadly classified into direct method and indirect methods.

Direct method: Isolation of causative microorganisms of sepsis from blood.

Indirect method: Over the last decade, a variety of laboratory tests have been developed to enhance the early and accurate identification and treatment of neonates with suspected sepsis. Those are haematological markers, serological sepsis markers and radiological evidences.

The gold standard method for diagnosis of neonatal sepsis is isolation of microorganism from blood. It is time consuming procedure usually takes more than three days for complete result and also requires well equipped laboratory and trained personnel for better results. Hence alternative fast diagnostic test of serological markers enabling earlier detection of neonatal sepsis might be beneficial.

Increased neonatal mortality in neonatal sepsis scenario necessiates need of rapid and effective diagnostic test with 100% sensitivity and 100% specificity. Such an ideal test is not available at this point of time and yet to be invented.

Hence combinations of clinical signs, haematological and serological markers have been proved to be a useful strategy in the diagnosis of neonatal sepsis in resource poor settings.[6] Commonly used diagnostic haematological markers such as total leucocyte count, immature: total neutrophil ratio, platelet count, absolute neutrophil count and micro erythrocyte sedimentation rate are less sensitive and specific for diagnosing neonatal sepsis.

In recent days screening of serological markers such as C-reactive protein (CRP), and various cytokines have been suggested as being useful and more sensitive indicators for early identification of sepsis in neonates. The biomarkers are classified into early phase marker (Interleukin-6, Interleukin-8, Tumour Necrosis factor-α and Interferon-γ) mid phase marker (Procalcitonin) and late phase marker (C-reactive protein).[7]

C-reactive protein (CRP) is an acute-phase protein synthesized by the liver within six hours after the onset of inflammation and tissue necrosis. Its rapid synthesis, short half-life and rapid decline with recovery, together with its increase in serious bacterial infections, have made the CRP test popular in the diagnosis of infections.[8] But it may also rise in systemic inflammatory conditions and giving rise to false positive results which limits its usage as specific diagnostic test.

Serum procalcitonin (PCT) is one of the most promising[9]. PCT, a 116-amino-acid protein with amolecular weight of 13 KDa, is the precursor in the synthesis of calcitonin (CT)secreted by the C cells of thyroid glandin normal situation but its levels may increaseduring septicemia, meningitis, pneumonia andurinary tract infection. This acute phase reactant has the characters of acute phase proteins, hormones and cytokines.[10]In sepsis, macrophages and the monocytic cells of the liver are involved in the synthesis of PCT.

Serum PCT concentration rises 2-4 hours after endotoxin injection, reaches its peak level right after 6 hours, maintains a plateau through 8 to 24 hours[11] and decreases to its normal level if the infection stimulus stops. It has been reported to be a reliable marker for severe bacterial infections and sepsis.[10]PCT levels increase in severesepsis and its plasma concentration is related to the patient's clinical condition. Serum PCT levelsappeared to correlate with the severity of microbialinvasionSeveral studies have reported on the usefulness of the quantitative measurement of PCT for an early diagnosis of sepsis in newborns.[12]Actually, definitive data are lacking, which can validate CRP and PCT as screening tools in the Emergency Department.

The aim of this study was to determine the diagnostic performance of PCT and CRP as early diagnostic markers for the detection of neonatal sepsis in the intensive neonatal care unit.

2.AIMS AND OBJECTIVES

- To detect the bacteriological profile of neonatal sepsis and antibiotic susceptibility pattern of the isolates in Tirunelveli Medical College.
- To determine the value of C-reactive protein and Procalcitonin in establishing the diagnosis of neonatal sepsis.
- To compare the efficacy of C-reactive protein and Procalcitonin with conventional blood culture method for the diagnosis of neonatal sepsis.

3. REVIEW OF LITRATURE

Neonatal sepsis is a clinical syndrome characterized by systemic signs of infection and isolation of a pathogen from blood, cerebral spinal fluid and from any other sterile site with in the first 28 days of life. Bacterial infection is a main cause of neonatal sepsis.

It includes various systemic infections of the newborn such as septicemia, meningitis, pneumonia, arthritis, osteomyelitis, and urinary tract infections. Superficial infections like conjunctivitis and oral thrush are not included under neonatal sepsis.[13]

In developed countries the mortality from neonatal sepsis is found to be in declining trend due to improved health care and appropriate use of antibiotics. But, the mortality due to neonatal sepsis is still high in developing countries accounting up to 50% of neonatal deaths.[14]

Rapid diagnosis and appropriate treatment is very essential in reducing morbidity and mortality associated with neonatal sepsis.[15] The empiric use of antimicrobial

treatment to all neonates presenting with clinical symptoms of sepsis practiced in resource poor settings in developing countries exposes neonates to adverse drug effects and promotes the development of drug resistant strains.[4]

3.1 Classification of neonatal sepsis

Neonatal sepsis is classified into early onset neonatal sepsis and late onset neonatal sepsis according to time of onset of signs and symptoms.

3.1.1 Risk factors for early onset neonatal sepsis are[16]

- Low birth weight – less than 2500 grams or prematurity
- Febrile illness in the mother with evidence of bacterial infection within two weeks prior to delivery.
- Foul smelling liquor and / or meconium stained liquor.
- Prolonged rupture of membranes - more than 24 hours.
- More than three vaginal examinations during lobour.
- Prolonged labour – more than 24 hours.
- Perinatal asphyxia.

3.1.2 Risk factors for late onset neonatal sepsis are[5]

- Low birth weight – less than 2500 grams or prematurity.
- Low Apgar scores.
- Low Immuoglobulin G (IgG) levels.
- Prolonged use of intravascular catheters.
- Treatment with steroids.
- Total parenteral nutrition.
- Delayed enteral feedings.
- Male sex.

The incidences of neonatal sepsis are more common in male infants compared to female infants. This male predominance may be due to X-linked immunoregulatory gene factor contributing to increased host's susceptibility to infections in male neonates.

A study conducted in Nepal during period of two years from July 2007 to June 2009 by YR Khinchi AK *et al*, found that among 175 neonatal sepsis cases, 65.1% were male infants and 34.9% were female infants.[17]

In a study conducted by RekhaSriram*et al* , Sri DevarajUrs Medical College, Karnataka, India, out of 115 clinically suspected sepsis cases, 76 (66.1%) were

males and 39 (33.9%) were females. In this study 48 cases were positive for blood culture. Among culture positive cases, male infants were 35 (60.3%) and female were 23 (39.7%). Male infants were predominantely affected compared to female infants with a ratio of 1.5:1.[18]

A study conducted by SucilaThangam*et al* found that among 50 clinicallysuspected neonatal cases, EONS cases were 58% and remaining 42% were undercategory of LONS.[19]

Another study from Tanzaia, during March to November 2009 done by NeemaKayange*et al* reported that 60% cases were LONS and 40% cases were EONS among 300 clinically suspected neonatal sepsis cases.[20]

Recent study done by Flora Chacha*et al at* Catholic University of Health and Allied Sciences, Tanzania during October 2013 to April 2014 revealed among 305clinically suspected sepsis cases, 224 cases (73.4%) were under the age group of 0-3 days (less than 72 hours).[21]

The incidence of neonatal sepsis is inversely associated to gestational age. A study conducted by Seo*et al.* revealed that increased sepsis incidence of 16.6% in preterm neonates with a gestational age less than 28 weeks and only 0.6% of incidence of neonatal sepsis in term neonates.[22]

A study conducted by Rabindra N Misra*et al* over a period of one year from October 2010 to October 2011 highlighted that out of 115 clinically suspected neonatal cases, 75 were found to be culture positive cases. Among these, 75% of proved sepsis cases were preterm and low birth weight neonates. Higher incidence of sepsis in preterm and low birth weight neonates are due to inherent deficiency of both humoral and cellular immunity during the first week of life.[23]

Long term complications are more in neonatal sepsis of preterm infants compared to term infant with neonatal sepsis. A study of Rachel E *et al* in Brazil (study period 2012-2013) reported that neurological complication are 2.5 times more in preterm infants with very low birth weight compared to term infants.[24]

3.2 Causative organisms:

The causative organisms include a wide variety of gram positive and gram negative organisms. These include *Staphylococcus aureus*, Coagulase negative *Staphylococcus*(CONS*),Escherichia coli (Ecoli), Listeria monocytogenes, Klebsiellapneumoniae,* Group B *streptococcus(*GBS*), Acinetobacter*, *Serratia*, *Pseudomona*s, *Haemophilusinfluenzae*, *Enterobacter, Candida* and anaerobes. The common bacterial isolates for early neonatal sepsis are *Klebsiellapneumoniae, Staphylococcus aureus* and *Escherichia coli.* In developing countries,

Staphylococcus aureus is found to be a significant neonatal pathogen isolated from 8-22% of blood culture.[25]The etiological bacterial agents differ from developed to developing countries. In 1990, Group B *Streptococci* was the commonest organism for EONS in developed countries. *Escherichia coli* was second common organism.[26] Following Group B *Streptococci* prophylaxis, developed countries identified gram negative enteric pathogens as the most common causative organism.[27]

A study done by Dar es Salaam *et al* at Muhimbili National Hospital in Tanzania found *Staphylococcus aureus* and *Klebsiella*species to be the most common causes of neonatal sepsis.[28]

A study carried out in China by Xin-Chuan Chen *et al* found gram positive organism (77.4%) was predominant in neonatal sepsis in Sichuan University.[29]

NeelamKaistha*et al* conducted a study at Government Medical College Hospital at Chandigarh (study period July 2003 to October 2007) which revealed that gram negative organism were responsible for 80.40% neonatal sepsis among 296 blood culture positive cases.[30]

A study conducted by RajlakshmiViswanathan*et al,* in Institute of Post graduate Education and Research and Seth SukhlalKarnani Memorial Hospital Kalkata reported gram negative organism (58%) mainly enteric gram negative bacteria as

causative organism for neonatal sepsis among 216 neonatal blood culture samples.[31]

A study done by NeemaKayange*et al* found that among 149 culture positive cases, 61.1% of sepsis were due to gram negative bacteria. Common bacterial isolates were *Klebsiellapneumoniae, Escherichia coli* and *Staphylococcus aureus.* More than 50% of *Klebsiellapneumoniae, Escherichia coli* and other gram negative organism were resistant to third generation cephalosporins with majority of them Extended Spectrum Beta Lactamase (ESBL) producer. Among 32 *Staphylococcus aureus* , 28% of isolates were Methicillin Resistant *Staphylococcus aureus.*[20]

Based on study conducted in North India by Kaistha N *et al* found that 88% gram negative isolates were resistant to gram negative cephalosporin.[32]

Based on the study conducted by Emily J Welson*et al* at Centres for Disease Control and Prevention, Atlanta and various centre in USA, reported that most common organisms were group B Streptococci (37.8%), *Escherichia coli* (24%), viridans Streptococci (18%), *Haemophilusinfluenzae*(4%) and *Staphylococcus aureus* (4%). This study showed that group B *Streptococci* as the commonest pathogen of early onset neonatal sepsis.[33]

3.3 Pathogenesis of neonatal sepsis:

The normal amniotic membrane, placenta and amniotic fluid by itself serves as a natural protective barrier against infection for inutero fetus by exhibiting anti-microbial effects.[34] Fatal systemic bacterial infection may occur in preterm and also in term infants with the neonates presenting symptomatic at birth itself. This infers bacterial infection may occur at inutero stage. Bacterial infection like *Listeria monocytogenes*is an example for transplacental route of infection affecting neonates via maternal circulation.[35]

3.3.1Pathogenesis of intrauterine infection

Clinical or subclinical maternal infection with agent like cytomegalovirus, Treponema pallidum, Toxoplasma gondii, rubella virus, varicella virus and parvovirus B19 are transmitted to fetus by transplacental transmission. Transplacental infection may occur at any time during pregnancy. Signs of transplacental infection may be noticed immediately after delivery or at later periods variable from months to years. Transplacental infection may result in early spontaneous abortion, congenital anomalies, intra uterine growth retardation, premature birth, stillbirth or asymptomatic persistent infection with sequelae later in life.

First trimester infection may affect organogenesis causing congenital anomalies. Example is congenital rubella. Last trimester infection frequently results in intra partum acquirement of pathogens. In third trimester infection, clinical manifestation occurs sometime after birth.

3.3.2 Pathogenesis of ascending bacterial infection:

Intact amniotic membrane serves as natural protective barrier against the inutero stage infection of fetus. Some anaerobic and aerobic microbes are found as normal inhabitants of birth passage and those microbes may at times cause ascending bacterial infection.

Chorioamnionitis is microbial infection of amniotic fluid and chorioamniotic membrane. Maternal fever, lower abdominal pain, foul smelling vaginal discharge/amniotic fluid, maternal leukocytosis, maternal and/or fetal tachycardia are main signs and symptoms of chorioamnioitis. Prolonged rupture of membrane (more than 24 hour)may cause chorioamionitis and inturn ascending infection. Bacterial colonization only does not cause neonatal infection. Prematurity, underlying illness, inoculum size, virulence of infecting organism, the innate immune system and transplacental antibodies are main factors for early neonatal infection. Aspiration or ingestion of bacteria in amniotic fluid, endotracheal

intubation, insertion of an umbilical vessels catheter are other factors result in early neonatal sepsis.

A study conducted by Pyati*et al* detected Group B *Streptococci* sepsis among 3000 newborn infants. Nearly all with early neonatal sepsis and a birth weight less than 2000 gram presented with symptoms less than one hour after birth, whereas more than two-thirds of those with a higher birth weight developed symptoms later than four hours.[36] These findings indicate that preterm neonates may be exposed to GBS in utero, whereas term neonates often may be exposed during the passage through the birth canal and by aspiration of contaminated amniotic fluid, or by bacteria penetrating through injured skin or natural body openings. In most cases this colonisation proceeds without causing disease. The mechanism by which bacterial colonisation converts to invasive disease is not fully understood, but it is mainly depends upon bacterial virulence, maternal immunological factors, and the competence of the neonatal immune system.[37,34]

In the study of Seo*et al*, on EONS (culture proven or clinical) found clinical chorioamnionitis in 34.7% of preterms with a gestational age less than 28 weeks, among 22.2% with gestational age 31-33 weeks, and 9.1% of term neonates. Compared to noninfected deliveries, clinical chorioamnionitis increased the risk of early-onset neonatal sepsis 8 to 10 times.[22]

3.3.3 Pathogenesis of late onset post natal infection:

In late-onset neonatal sepsis, cases are less likely to have a history of obstetric complications, and may be infected mainly with nosocomial acquired organisms. Post natal infection occurred by direct contact with hospital personnel, the mother, and other family members or from inanimate sources such as contaminated equipment.[34]

3.4 Immunity:

Both term and pre term infants have decreased function of neutrophils, and decreased complement level. These factors are mainly responsible for neonatal infection. Preterm babies have low immunoglobulin concentration compared to term babies. Maternal IgG antibody is actively transported to fetus through the placenta. These IgG levels are directly proportional to gestational age. Ig M and IgA are not transferred across the placenta, although a fetus can synthesize IgM and IgA in response to intrauterine infection. Neonatal tetanus and Group B Streptococci infections are prevented by maternal Ig G antibody. IgM antibodies are mainly synthesized against gram negative enteric pathogens. Usually newborn infants lack antibody mediated protection against *Escherichia coli* and other Enterobacteriaceae.

3.5 Complement:

The complement system facilitates bactericidal activity against certain organism such as *Escherichia coli* and functions as an opsonin with antibody in the phagocytosis of bacteria such as Group B Streptococci. Maternal complement does not cross placenta. A fetus begins to synthesis complement components in first trimester itself. Usually term newborn infants have slightly diminished classical pathway complement activity and moderately diminished alternative pathway activity. In comparison to term infant, preterm infants have low levels of complement components and less compliment activities. These deficiencies contribute to diminished complement derived chemotactic activity and to a diminished ability to opsonize certain organisms in the absence of antibody. In neonatal infant, opsonization of *Staphylococcus aureus* is normal, but various degrees of impairment have been noted against *Escherichia coli* and *Group B Streptococci*.

3.6 Neutrophils:

Qualitative and quantitative deficiencies of the phagocyte system mainly contribute to neonatal sepsis.

Causes for increased susceptibility of neonates to infection are stated as

1. Abnormal Neutrophil migration at birth in both term and preterm infants. Specifically telling decreased adhesion, aggregation and deformability of neutrophils may delay prompt response to infection.

2. Abnormal expression of cell membrane adhesion molecules β2 integrins and selectins and abnormalities in neonatal neutrophil cytoskeleton leads to abnormal chemotaxis and hence impair response to infection.

3. Impaired oxidative respiratory burst of neonatal neutrophils aids in increased risk of sepsis.

4. Decreased storage pool of neutrophil - Frequently noticed neutropenia in preterm and intra uterine growth retarded infants attributes to the increased risk for sepsis.

3.7 Monocyte ‑Macrophage system:

Prime functions of activated macrophages include antigen presentation, phagocytosis and immune modulation. Although count of monocyte in neonatal blood is normal, function of macrophages such as impaired chemotaxis increases risk of sepsis.

3.8 Natural killer cells:

Natural killer cells (lymphocyte sub group) are cytolytic against cells infected with pathogens. These cells also lyse antibody coated cells and this action is called antibody depended cell mediated cytotoxicity (ADCC). Natural killer cells appear early in gestation and are of equivalent numbers as in adults. However diminished cytotoxic activity and ADCC predispose to increased susceptibility of neonates to sepsis.

3.9 Cytokines and acute phase proteins:

Cytokines are endogenous chemical mediators that carry information between different cells and are important factors in the human inflammatory response. They are regulated by a complicated web of regulatory mechanisms including several different cell types. In case of infection, both pro-inflammatory and anti-inflammatory cytokines are upregulated according to a specific time schedule, and so by studying this upregulation in blood samples we can conclude whether systemic inflammation is present or not. Tumor necrosis factor-α (TNFα), Interleukin-1 (IL-1), IL-4, IL-6, IL-8, IL-10, IL-12 and platelet-activating factor are important chemical mediators released in various inflammatory reactions. Potential marker for bacterial neonatal sepsis, pneumonia and necrotizing enterocolitis are TNFα, IL-6 and IL-8.

Innate immunity also plays an important role against an infectious agent which is due to nonspecific cellular and humoral response. Recognition of pathogens is initiated by soluble components in plasma (including mannose binding lectin) and by recognition of receptors monocyte and other cells.

3.10 C-Reactive Protein:

Tillet and Franchis of Rockefeller University were firstly described C – reactive protein. They demonstrated a precipitation reaction with polysaccharide fraction C from the peumococcal cell wall and serum of patient suffering from peumococcal pneumonia. Serum of healthy controls and some pneumococcal pneumonia recovered patients does not show this precipitation. In interpretation of the fact that the polysaccharide fraction was a protein, the C-reactive component in the serum was named C-reactive protein.[38] In 1950, CRP had been detected in more than 70 various disorders including acute bacterial, viral, and other infections, as well as noninfectious diseases such as, rheumatic disorders, acute myocardial infarction, and various malignancies. All of these disorders of different etiology had in common the factor of inflammation and/or tissue injury. Increased serum level of CRP is very early and sensitive response to most of microbial infections.[39]

C- reactive protein (CRP) is one of the acute-phase proteins. It belongs to the pentraxin family of ligand-binding and calcium-dependent plasma proteins. In acute infection, serum level of CRP increased up to 50 to 100 mg/L. But in chronic

condition like rheumatoid arthritis and atherosclerosis its level generally remains below 10 mg/L.[40]

3.10.1 Synthesis and metabolism:

Hepatocytes are main site for production of CRP. CRP synthesis and secreted depends upon various response to cytokines such as Interleukin-6, Interleukin-1 and Tumour Necrosis Factor-α (TNF-α). CRP is primarily derived via IL 6-dependent hepatic biosynthesis. Increased CRP level in neonate always represents endogenous synthesis. CRP passes through the placenta is very very minimal. Only single stimuli enough to hepatic synthesis of CRP, that increase the serum concentration above 6mg/l by about 6 hours and peaking at around 48 hours.[41] The stimulatory effects of cytokines on the production of acute phase proteins increased by glucocorticoids.[42] Insulin, on the other hand, decreases their effects on the production of some acute phase proteins.[43,44]

3.10.2 CRP detection method:

A large number of methods are available for the detection of CRP and estimation of CRP level in the serum. Even though, nephrometry, electroimmunoprecipitation assay and immunometric assay are sensitive and quantitative method for the estimation of CRP. These methods have complicated procedure, so these tests done only well-equipped laboratories only.[39] Latex agglutination test is the alternative

test, it have a quiet sensitive, rapid method to detect serum CRP in qualitatively and semi quantitatively. The serum CRP concentration of 6mg/L or more was considered as positive.

3.10.3 Function of C-reactive protein:

The main receptor to CRP is phosphocholine. Phosphocholine is found in lipopolysaccharide of bacterial cell wall and in most of biological membrane. CRP and phosphocholine binds first, after that CRP is recognized by the complement system. CRP activates the complements system and promotes the phagocytosis by neutrophils and macrophages. Then CRP initiates release of proinflammatory cytokines.[45,46]

The sensitivities and specificities of CRP assay in the detection of neonatal sepsis using culture as a gold standard. Sensitivity of CRP is more important than specificity in detection of EONS and LONS.

3.10.4 Condition where CRP is elevated:

CRP level is increased in some acute conditions such as bacterial infection, bacterial endocarditis, pneumococcal pneumonia and acute rheumatic fever. CRP level is increased in more number of chronic condition like polyarthritis nodosa, rheumatoid arthritis, systemic lupus erythematosis, Inflammatory bowel disease, acute myocardial ischemia and malignancies etc.[44]

A study conducted by Benitz MD *et al* from Stanford University of Medicine shows 54.6% of sensitivity on proven neonatal sepsis and 65.5% of sensitivity in probable neonatal sepsis among 1002 infants. The positive predictive value was 99.7% and negative predictive s for CRP was 98.7% for conformed neonatal sepsis. [47]

Laborada G *et al* revealed that during study (2003), out of 105 neonatal sepsis cases blood culture tested 48 cases were positive by automated technique. This study also shows 69% sensitivity , 96% specificity, 93% positive predictive value and 80% negative predictive value.[48] A similar study by Doellner H *et al* reported that CRP sensitivity 63%, specificity 97%, positive predictive 83% and negative predictive value 91%. Doellner H et al study also include 36 samples are positive among 253 blood culture.[49]

According to a study conducted by Franz A R *et al* revealed that 46 cases are culture positive among 162 neonates with suspected sepsis and also reported 28% of CRP sensitivity, 97% of specificity, 81% of positive predictive and 77% of negative predictive value.[50]

Neonates with septicemia due to gram negative organism have higher serum CRP level than gram positive organism.[51]

Recent study by Flora Chacha *et al* highlighted that out of 305 samples received from clinically suspected cases of neonatal over a period of 2 years, 104 cases

showed CRP positive. This study also revealed CRP sensitivity 90% , among these 75% higher sensitivity for gram negative septicemia compare to 50% sensitivity for gram positive septicemia.

A study conducted in Thailand by Nuntnarumit P *et al* found that serial quantitative CRP measurement were found to have better predictive than complete blood count with 100% sensitivity, 94% specificity, 91% positive predictive and 100% negative predictive. [52]

A study was done in Rawalpindi Pakistan by Khassawneh M *et al,* comparing CRP, absolute neutrophil count and I/T ratio, CRP was found to have a specificity of 95% in diagnosing neonatal sepsis followed by absolute neutrophil count.[53]

A study conducted by Kohli-Kochhar R *et al,* in Port Harcourt Nigeria, the sensitivity, specificity, positive predictive value and negative predictive of serial CRP measurements were found to be 74.0%, 74.1%, 68.0% and 79.0% respectively in the diagnosis of neonatal sepsis using blood culture as the gold standard.[54]

In a study done by Hofer N *et al,* comparing CRP, interleukin 6 and immunoglobulin M; revealed that CRP was the best among the three with 95% sensitivity and 98% NPV in the diagnosis of early gram negative sepsis.[55]

Serial serum CRP measurements taken between 24 and 48 hours after the onset of infection have been found to have high sensitivity for probable septicemia. Hence

serial CRP is suggested for the diagnosis of neonatal sepsis to predict early infection.[56] The neonates who were admitted with clinical features of neonatal sepsis and started on empirical treatment with antibiotics following the negative results of CRP, the physician can stop the antibiotics thus can minimize antibiotic exposure and shorten hospital stays. The diagnostic value of serial measurements of serum CRP levels can also be used for monitoring the severity of sepsis and improvement after initiation of treatment.[57]

3.11 Procalcitonin:

Procalcitonin is an another acute phase protein which is made up of 116 amino acids. It is a precursor of calcitonin. Within 6-8 hours of bacterial infection, bacterial endotoxin stimulates monocytes and hepatocytes which produce procalcitonin. Its level reaches peak at 6 – 8 hours, and stays minimum for a day. Its half-life is upto 30hours. Procalcitonin level is increased during infection in neonates, children and adults. Serum procalcitonin level is more increased in bacterial infection than in viral infection. In early neonatal bacterial infection, procalcitonin is more sensitive than CRP.[58]

3.11.1 Structure and production of proclcitonin:

PCT is one of a group of peptides in the calcitonin super-family of peptides. The PCT peptide has an approximate molecular weight of 14.5 kDa and consists of a sequence of 116 amino acids. PCT is encoded by the Calc-1 gene located on chromosome 11p15.[59] The peptide has three regions: the PCT amino terminus, the mature calcitonin segment, and the carboxyl-terminus called katacalcin.

In the absence of infection, the production of PCT outside of the neuroendocrine cells of the thyroid and the lung is suppressed. In the presence of sepsis, all tissues produce PCT. Because of this dual role, PCT is considered a “homokine”. Homokines can either act as a hormone as in the normal physiologic state or as a cytokine during inflammatory processes.[60]

As with other cytokines, there is little intracellular storage of PCT during sepsis. While synthesis of PCT is necessary for the production of calcitonin, animal studies have shown that increased concentrations of PCT may have lethal effects during sepsis. Administration of PCT to septic hamsters with peritonitis doubled the death rate to over 90%. Immunoneutralization of PCT by the administration of antiserum in septic hamster and pig studies led to increased survival of these animals[60]

3.11.2 Procalcitonin detection methods:

Quantitative and qualitative (semi-quantitative) assays available for measuring PCT

1. Qualitative tests: rapid test strips for point-of-care testing (results available in < 30 minutes)

2. Quantitative tests: use luminescence immunoassay, ELISA (results available in a few hours).

3.11.3 Procalcitonin levels and interpretation:

Normal:<0.1ng/mL (infants>72 h – adults)

Suspected lower respiratory tract infection

0.1–0.25 ng/mL – Low likelihood for bacterial infection; antibiotics discouraged.

>0.25 ng/mL – Increased likelihood for bacterial infection; antibiotics encouraged.

Suspected sepsis: Strongly consider initiating antibiotics in all unstable patients.

0.1–0.5 ng/mL – Low likelihood for sepsis.

>0.5 ng/mL – Increased likelihood for sepsis.

>2.0 ng/mL – High risk of sepsis.

>10 ng/mL – Septic shock .

A study done by Cetinkaya M *et al*, during the period of 2008-2009 found that the serum procalcitonin levels were higher in the neonatal septic cases compared with

the non-septic cases. This also revealed procalcitonin and CRP had sensitivity of 97%, specicity of 91%, positive predictive value 96% and negative predictive value of 87%. The inference was procalcitonin more than 2.3 ng/ml or CRP more than 30 mg/l denotes a possibility of EONS and LONS. In such condition antimicrobial treatment need to be carried over in the absence of positive culture.[51]

In a recent study Koksal et al concluded that serum procalcitonin level was superior to serum CRP level in terms of early diagnosis of neonatal sepsis, in detecting the severity of the illness and in evaluation of the response to antibiotic treatment.

Athhan et al in their study revealed that at 7th day of therapy neonates who had achieved clinical recovery had a significant decrease of procalcitonin levels compared to the initial values.

Carol et al in their study showed that procalcitonin is more sensitive than the CRP in the diagnosis of septicemia, meningitis and urinary tract infection.

Kawezynski and Piotrowski analyzed inflammatory parameters in 48 newborn infants suffering nosocomial sepsis admitted to the intensive care. They obtained samples for PCT and CRP levels just at time of onset of the signs and 24 hours later. At the onset of gram negative sepsis 14 of 17 contaminated newborns had significantly increased PCT and CRP levels, but at the onset of gram positive sepsis only 18 from 31 neonates with positive blood culture had

increased CRP level and 28 of them had elevated concentration of serum PCT. These differences were statistically significant

3.12 Clinical features of neonatal sepsis:

Clinical features of neonatal sepsis are mainly variable. Clinical features of neonatal sepsis is divided into non-specific features and specific features.

Non-specific features: The earliest signs of sepsis are frequently subtle and nonspecific.

Clinical diagnosis needs a high index of suspicion for early diagnosis.

Clinical features are “hypothermia or fever, lethargy, poor cry, refusal to suck, poor perfusion, prolonged capillary refill time, hypotonia, absent neonatal reflexes, brady/tachycardia, respiratory distress, apnea and gasping respiration, hypo/hyperglycemia and metabolic acidosis”.

Early manifestation of neonatal sepsis may involve only one system and present with limited symptomatology. Initial signs and symptoms of neonatal sepsis are temperature instability (hypothermia or fever), refusal of feeding and edema.Signs and symptoms related to respiratory system are apnea, tachypnea, grunting, cyanosis, retractions of chest wall and nasal flaring. Main signs and symptoms related to cardio vascular system are pallor, cold and clammy skin, tachycardia (more than 160 beats /min) or bradycardia (less than 100 beats /min)

and hypotension. Signs and symptoms related to central nervous systems are lethargy, irritability tremors, convulsion, abnormal moro reflex and hypotonia. Abdominal distension, vomiting, diarrhea and hepatomegaly are main signs and symptoms related to gastro intestinal tract. Oliguria is main symptom related to renal system. Signs and symptoms related to haematologic systems are jaundice, splenomegaly, pallor, petechial purpura and bleeding. Signs and symptoms related to skin and soft tissue are impetigo, omphalitis, scalp abscess, fascilitis, adenitis and abscess of cystic hygroma. Most of the times, various non-infectious diseases can coexist with neonatal sepsis, which in turn makes sepsis diagnosis a tough one. Surfactant deficiency leading to respiratory distress syndrome can coexist with bacterial pneumonia.[61]

3.13 Clinical criteria for neonatal sepsis:

3.13.1 Integrated Management of Childhood Illness criteria:

“Tachypnea (> 60 breath per minute), nasal flaring, increased chest retraction, grunting, nasal flaring, bulging fontanel, pus draining from ear, redness around umbilicus, temperature instability (>37.7˚C or <35.5˚C), lethargic, reduced movements, not able to feed and convulsions” are components of integrated management of childhood illness criteria for neonatal sepsis.

3.13.2 WHO criteria:

"Convulsion, tachypnea (> 60 breath per minute), severe chest retraction, temperature instability (>37.7˚C or <35.5˚C), lethargic, reduced movements, not able to feed, crepitation and cyanosis" are main components of WHO criteria for neonatal sepsis.[62]

A study conducted by Jaswal RS *et al*, in Shimla medical collages India, found that the most frequent clinical presentation of neonatal sepsis were respiratory distress, lethargy and jaundice with combined frequency of 40% followed by fever and poor feeding.[63]

Tanzania based study by Arif SH *et al*, stated the most common clinical presentation found in neonatal sepsis were fever reported in 91% of neonates, inability to breast feed, bulging anterior fontanelle , dyspnea, jaundice, and seizures. Few other clinical features of neonatal sepsis included abdominal distension, tachycardia, tachypnea, disseminated intravascular coagulopathy and abscesses.[64]

3.14 Diagnosis of neonatal sepsis:

Neonatal sepsis is a potentially dangerous and serious condition. That can cause increased mortality and morbidity very rapidly, if not treated correctly and quickly. The ideal test to confirm neonatal sepsis should have 100% sensitivity and 100%

specificity. But such a test is unlikely to be discovered till date, due to non-specific signs of neonatal sepsis. Many signs and symptoms of neonatal sepsis are also present in some of noninfectious conditions like acute respiratory distress, aspiration of amniotic fluid and hypoglycemia etc.[65] Hence it remains a challenge for physicians to correctly diagnose neonatal sepsis in a timely manner. So a rapid reliable diagnostic test for neonatal sepsis is essential in order to initiate treatment in suspected neonates on time so as to reduce associated morbidity and mortality.

In routine clinical practice, the recommended approach is to liberally start intravenous antibiotics and then perform a ruling out procedure that normally lasts for several days. If all the tests for neonatal sepsis are negative and neonate has recovered fully, the antimicrobials can be discontinued and infant can be discharged from the neonatal intensive care unit. This rule out procedure normally includes various cultures such as blood, cerebrospinal fluid, urine etc, x-rays and various markers of sepsis. If itwere possible to decrease the time taken by this investigation, the benefits would beobvious in terms of reduced costs of treatment, reduced infants suffering and reduced duration of antibiotics. So, there is a great need for specific and less time consuming diagnostic methods.

A study conducted by Ng PC *et al* in 2004, presented a list of 58 different laboratory tests that had already been evaluated as diagnostic tests for neonatal

sepsis.[66] Another study of review article by Pierrakos*et al* reviewed 3370 references covering 178 biomarkers.[67]

3.14.1. Blood culture:

Gold standard diagnostic test for suspected neo natal sepsis is blood culture. A small blood volume is enough for isolation of bacteriological agents as low as 0.2 to 0.5mls. But, increased blood volume 1 to 2mls is required for detection of low bacteraemia particularly where there is history of prior use of antibiotics. Venous blood is routinely used for blood culture. The skin should be prepared with disinfectant solution before veupuncture. But care must be taken; disinfectant solution does not harm skin of newborn infants.[68]

A study conducted in 1997 by Kellogg *et al* revealed that low level bacteremia was very common in infants. So they recommended a sample volume of 6 ml for to get optimize sensitivity. Though, this would represent around 4.5% of an infant's blood volume.[69]

Volume of blood needed for culture depends up methods. Automated blood culture systems such as BacT/Alert required small volume of blood such as 0.5ml only. But 1-2 ml needed for conventional method.

Schelonka RL *et al* found that, if one or two viable colony-forming units are in the blood inoculated into culture media, the BacT/Alert system will detect growth rapidly. Since there appears to be a sizable subset of neonates who are at risk of

sepsis with a colony count less than 4 CFU/ml, then a 0.5 ml inoculum of blood into the culture media is insufficient for sensitive and timely detection of bacteremia. One to two milliliters of blood should increase microorganism retrieval in the face of low-colonycount sepsis by conventional blood culture method.[70]

In developing countries, the conventional type of blood culture method is commonly used. Because it is less expensive to do when compared to automated culture systems. But the procedure is labour intensive and the yield is significantly low sensitivity than that of an automated system.[71] In developed countries, automated systems are mostly used. Main advantages of these techniques are the blood culture to be monitored continuously and resulting in a shorter time to identify a positive culture.[72]

A study conducted by Baltimore RS *et al* in Yale University of Medicine, New Haevan, USA "found ninety-four cases of non-GBS early-onset sepsis were detected between 1996 and 1999. The rate of GBS-related early-onset infection reduced from 0.61/1000 to 0.23/1000 births, but the annual rate of non-GBS sepsis remained steady, ranging from 0.65 to 0.68/1000 during the surveillance period. There was an increase in the proportion of *Escherichia coli* infections that were ampicillin resistant between 1996 and 1998, but the proportion decreased in 1999".[62]

A study (during 1998 to 2004) conducted by Ramesh Bhat *et al* highlighted that out of 2182 samples received from clinically suspected cases of early onset neonatal sepsis 389 (17.8%) showed positive blood culture.[26]

A study by SubhranshuSekarKar*et al* (2007–2010) revealed that, among 160 blood culture samples tested, 60(16.2%) were blood culture positive. This study was done at Hi-Tech medical college, Bhubaneshwar, Odisha.[73]

A study done by SucilaThangam*et al* in Tamiladu during April – September 2010 revealed 28% of positive blood culture in 50 samples. Based on the study conducted by Shrestha R K *et al* in Nepal medical college, Kathmandu, out of 120 suspected cases, 37 (30.8%) were found to be blood culture positive during the period of July 2011 to January 2012.[74]

Even though, if ideal blood volumes are used, blood culture has obvious limitations in sensitivity. A negative blood culture report alone cannot support withdrawal of antibiotic treatment if the neonate's clinical condition indicates ongoing sepsis. So blood cultures have limited sensitivity and this method is time consuming, and most microbiology laboratories will take one week for complete report.[75]

3.14.2 Haematological marker scoring system:

This test can be used for screening for neonatal sepsis. It can be performed easily

and it is readily available in most of the settings. It is usually a combination of various parameters from complete blood picture. Various parameters are

- "Total white blood cell count
- Absolute Neutrophil count (ANC)
- Immature Neutrophil : Total Neutrophil Ratio
- Platelet count
- Micro erythrocyte sedimentation rate
- C-reactive protein".[75]

In complete blood count, total leukocyte count, neutrophils and platelets are predictors of ongoing infection. Ongoing infection interpreted by extreme value of these parameters.[75,76]

Non-infectious causes such as asphyxia, maternal fever and post gestational age are elevating these parameters. This factors causes difficult in interpretation of results.[77]

Total white cell count alone could not be considered valid for confirmation of neonatal sepsis. The reasons being are its grossly varied values and narrow transition between normal and abnormal values. Neutropenia is seen more in sepsis rather than neutrophila. This neutropenia results from significantly raised adherence of neutrophils to surface of endothelial cells and its increased consumption at the area of infection.

Neutropenia also being noted in certain other condition such as inborn errors of metabolism and asphyxia neonatorum makes it of limited value as a sole marker of sepsis. Frequently neonatal sepsis is associated with low absolute neutrophils count, and high I/T ratio. Low white blood cell count are more helpful if obtained after 4 hours of life due to normal increase of white blood cell and neutrophil count after 6 hours of life.[78,79]

Hematological marker scoring interpretation:

- "Total leucocyte count: less than 5000/mm3 or more than 30000/mm3
- Absolute Neutrophil Count (ANC): less than 1000/mm3
- Immature / Total neutrophil ratio: more than 0.2
- Micro Erythrocyte sedimentation rate: more than 15 mm in hour.
- C-reactive protein: more than 1mg/dl".

If two or more abnormal hematological markers are present, it should be considered as a positive screen for neonatal sepsis. If the hematological marker score is negative, but clinical suspicion persists, it should be repeated within 12 hours. If the hematological markers are still negative, neonatal sepsis can be excluded. Presence of two abnormal hematological markers are associated with 93-100% sensitivity, 83% specificity, 27% positive predictive value and 100% negative predictive value in neonatal sepsis.[78]

Neonatal sepsis also causes thrombocytopenia, because of disseminated

intravascular coagulation and the damaging effects of endotoxin on platelets. Therefore combining the parameter of the complete blood count is the form of a Hematological markers Scoring System that has been suggested and can serve as a screening tools.[80]

3.15 Prevention strategies for EONS:

Prior administration of parental antibiotics to antenatal mothers prevents EONS by group *B Streptococci* to a great extent. Ampicillin or cefazolin are used as prophylactically four hour before delivery. In mother who have mild pencillin allergy, cefazolin may be administrated. In case of serious pencillin allergy, clinadamycin is the drug of choice. In clindamycin resistant cases, vancomycin is used as an alternative drug for prophylaxis.[81]

Indications for intranatal antibiotics are

- Positive antenatal culture for group B streptococci
- Premature rupture of membranes. (>18 hours)
- Previous infection with group B Streptococci.

3.16 Prevention strategies for LONS:

- To provide practical training for hand washing technique for entire health care team.
- To provide adequate soap and running water facilities.

- To prepare standard operating procedure for all invasive methods.
- To carry out regular meeting with infection control committee to monitor infection rate.
- To ensure the adequate of physicians and nurses per bed according to current recommendations.[81]

4. MATERIALS AND METHODS:

The present study was conducted at the Department of Microbiology , Tirunelveli Medical College, Tirunelveli from June 2016 to May 2017

4.1 Study group

A total of 100 clinically suspected sepsis cases in neonates (0 day to 28 days)

4.2 Inclusion criteria

Neonates who were admitted in Neonatal Intensive Care Unit at Tirunelveli Medical College with signs suggestive of sepsis, or those who developed signs of sepsis while they were in the ward.

4.3 Exclusion criteria:

Neonates who were on antibiotics,

Neonates who had birth asphyxia and aspiration syndromes,

Neonates who had congenital anomalies and inborn errors of metabolism.

4.4 Ethical clearance

Ethical clearance was obtained from the college ethical committee before the commencement of the study.

4.5 Consent

Informed consent was obtained from reliable informants of neonates who participated in the study.

4.6 Proforma:

The proforma was filled with the details like name of mother of neonates, age (in hours or days), sex, weight of neonates at time of birth, gestational age (in weeks), mode of delivery and clinical diagnosis and other parameters relevant to the present study.

4.7 METHODS

Blood samples were taken from 100 clinically suspected neonatal sepsis and were processed for blood culture, detection of serum level of CRP by latex agglutination test and detection of serum level of PROCALCITONIN by ELISA.

4.7.1 Blood collection method:

Ideal blood sample collection should be done before initiation of anti-microbial agents.

Volume of blood needed for culture:

Amount of blood needed for cultures for neonates is significantly lower than that needed for adults because neonates tend to have a higher concentration of bacteria in their bloodstream than adults. Hence 2ml of blood was usually considered as the standard volume of blood adequate to detect bacteremia in neonates.

- Proper aseptic precautions were undertaken during blood specimen collection to avoid sample contamination.

- With clean gloved hands, preliminary aseptic precautionary steps like cleansing the venipuncture site with 70% ethanol and 2% tincture iodine and proper drying were followed.
- Then using a 2ml syringe with a 28G needle about 2-3 ml of blood was aspirated. Immediately and without changing or contaminating the needle 2 ml of blood sample was transferred into the top of the blood culture bottle that contains 20ml brain heart infusion broth. (HiMedia, India)
- Another 1 ml of blood was collected in serum separating vial.
- Sharps were disposed in a sharps container.
- The culture bottle was gently mixed and labeled.
- The inoculated bottles were sent to the laboratory immediately.
- The collected samples were subjected to various laboratory studies.

4.7.2 Storage of serum sample:

Blood samples were centrifuged within 30 minutes of collection. Serum samples were immediately tested for CRP by latex agglutination method and then stored for Procalcitonin ELISA at -20°C.

4.7.3 Blood culture processing procedure:

Inoculated culture bottle was incubated at 37˚C for up to 7 days. Subsequent sub culture was done in solid agar plates such as blood agar plate, chocolate agar plate, Mac conkey agar plate and nutrient agar plate after 24 hours and 72 hours with last

subculture being done after seven days. The subcultured blood agar plate, Mac Conkey agar plate and nutrient agar plates were incubated aerobically and chocolate agar plate was incubated in carbon dioxide atmosphere for 24 hours.

|The isolates were routinely identified by standard bacteriological techniques.

4.7.4 ANTIBIOTIC SUSCEPTIBILITY TESTING:

The antibiotic susceptibility testing was done in all isolates by Kirby Bauer disc diffusion method according to the CLSI guideline.

Kirby-Bauer's disc diffusion method:

About 3-5 colonies of the test organism were inoculated in 2 ml of peptone water and incubated for 2-4 hours at 37˚C. The turbidity of the inoculum was adjusted to 0.5 McFarland standards ($1.5x10^8$ CFU/ ml). A sterile cotton swab was soaked in the inoculum and a lawn culture was made on to the Muller-Hinton agar (MHA). By rotating the swab against the inner side of the test tube, excess broth was expressed. The panel of antibiotic discs was applied and incubated at 37˚C for 18-24 hours. The zone size was recorded and interpreted as per the CLSI guidelines.

The three interpretive categories are described as follows.

Susceptible:

This indicates that the recommended antibiotic in appropriate dose for recommended period is the appropriate agent for treating the infection.

Intermediate:

This indicates that the tested organism may be inhibited by possible concentrations of certain drugs if higher concentrations of the drug can be used safely.

Resistant:

The antibiotic tested may not be an appropriate choice for the infection against the tested organisms either they are not inhibited by the concentration of the drug normally achievable with the recommended dose or because the test result vastly correlates with a resistance mechanism.

4.8 Serum CRP level detection by latex agglutination test:

All the 100 samples were tested for CRP detection by latex agglutination test with the help CRP test kit of AGAPPE DIAGNOSTICS, INDIA

4.8.1 Principle:

Specially selected polystyrene latex particles are coated with monospecific goat anti human CRP antibodies. When a serum positive for C - reactive protein is mixed with the latex reagent, a positive result is indicated by a distinctly visible agglutination of the latex particles in the test cell of the slide used. In specimen negative for C –Reactive Protein, the latex remains in a smooth suspension form in the test cell.

4.8.2 Materials provided:

- Latex reagent for tests – Suspension of polystyrene latex particles, coated with monospecific goat anti-human CRP antibodies.
- Positive Control serum – 0.5ml
- Negative Control serum – 0.5 ml
- Reaction slide – 1no
- Applicator sticks- 50 nos
- Serum droppers – 50 nos
- Rubber teat – 1 no

4.8.3 Storage

C-Reactive Protein latex agglutination kit was stored at 2-8 ˚C.

4.8.4 Test procedure:

- The latex reagent, controls and serum specimens were brought to room temperature. The antigen suspension was mixed thoroughly prior to use.
- One drop each of patient serum, positive and negative control sera were placed in respective cells of the test plate.
- Then one drop each of CRP latex reagent was added to each of these sera.
- The sera and latex reagent were mixed with separate mixing sticks and the

Fluid spread over the entire area of the particular cells.

- The test slide was tilted back and forth for two minutes so that the mixture rotates slowly inside the cells .
- At the end of two minutes the results were read under bright light.

4.8.5 Interpretation of results:

Strong Positive – Distinct coarse agglutination occurs within 0.5 minute.

Weakly Positive – Fine agglutination usually taking full 2 minutes.

Negative - No agglutination.

Distinct agglutination indicates CRP content of more than 6 mg/litre in undiluted serum specimen.

4.9 Serum Procalcitonin level detection by ELISA:

All the 100 samples were tested for Procalcitonin using ELISA with the help of HUMAN PROCALCITOIN ELISA KIT (SINCERE BIOTECH, Beijing, China).

4.9.1 Principle of the method:

Standard and Samples are aspirated into the wells and Human PCT present in them is bound to Human PCT monoclonal immobilized antibodies ,which pre-coated onto 96-well plate.The Biotinylated detection antibodies are added to the

wells and then followed by washing with PBS or TBS buffer. After washing away unbound Biotinylated antibody, Avidin-Biotin-Peroxidase Complex is added to the wells.The wells are washed again, a TMB substrate solution is added to the wells and the color changes after adding acidic TMB Stop solution. The intensity of the color is proportional to the amount of Human HPV bound in samples and measured at 450nm±10nm. The absorbance of thecolour complex is then measured and the generated OD values for each standard areplotted against expected concentration forming a standard curve. This standard curve canthen be used to accurately determine the concentration of Procalcitonin in any sample.

4.9.2 Materials provided:

Precoatedmicrotitre late 8×12

Standards (frozen dried) 2vials

Biotinylated detection antibodies 1 vial*120ml

Avidin biotin peroxidase complex 1 vial*120ml

TMB colour developing reagent A 1vial*10ml

TMB colour developing reagent B 1vial*1.5ml

Sample diluent buffer 1vial*14ml

ABC diluent buffer 2vial*12ml

Antibody diluent buffer 1vial*12ml

TMB stop solution 1vial*10ml

TMB wash buffer 1vial*20ml

4.9.3 Materials required:

1）Microplate reader (450nm detection wavelength filter, 570nm or 630nm correction wavelength filters)

2）Beakers, flasks, cylinders necessary for preparation of reagents

3）Clean benches, Incubator(37℃), Refrigerators (4℃, -20℃), Low Temperature Centrifuge

4）High-precision single-channel and multi-channel Pipette and disposable Tips.

5）Polypropylene tubes for diluting and aliquoting Standards

6）Distilled or de-ionized water

7）Absorbent paper for blotting the microtiter plate

8）Automated or manual microplate washer

4.9.4 Kit storage:

All the components in the kit should be stored up to 1 year at -20℃ and 3 months at 2-8℃.

4.9.5 Assay procedure:

- Add 100µl of each, standard and diluted sample to appropriate number of wells.
- Plates were sealed and incubated at 37°C for 90min
- The wells were washed 3 times with diluted washing solution using an automatic washer.
- 100µl of Biotinylated anti- Human PCT antibody working solution was added to into each well
- Plates were sealed and incubated at 37°C for 60min
- The wells were washed 3 times with diluted washing solution using an automaticwasher.
- 100µl of TMB working solution was added to each wells and incubated at 37°C for 15min away from the light
- 100µl of TMB stop solution was added to each wells to stop the reaction.
- The absorbance of each well was read within 30 minutes at a wavelength of 450nm.

4.9.6 Data analysis:

Absorbance values of standards, controls and samples were calculated. Linear standard curve was generated by bloating the OD value of each standard on the vertical axis versus the corresponding Procalcitonin standard concentration on the

horizontal axis. The amount of Procalcitoninin each sample was determined by extrapolating OD values against Procalcitoninstandardconcentrations using the standard curve.

5. RESULTS

5.1 The Study Group

A total of 100 neonates (0 to 28 days) who fulfilled the criteria of clinicallysuspected sepsis were analyzed. This study was conducted at the Department ofMicrobiology , Tirunelveli Medical College Hospital, Tirunelveli over a period of

one year from June 2016 to May 2017

5.2 Statistical Analysis

All the results obtained were analyzed statistically for their completeness ,consistency and accuracy by the parameters like mean and percentages. Kappa value was calculated to measure the degree of agreement between three diagnosis methods- Blood culture with CRP and Blood culture with Procalcitonin. The correlation of serum CRP level and Procalcitonin level with blood culture for neonatal sepsis was comparedstatistically and results were analyzed by IBM SPSS Statistics 20. Chi-square test and Fisher Exact test were used in calculating the P-value. The P Values of less than 0.05we**r**e considered as statistically significant ($P<0.05$).

Result Analysis:

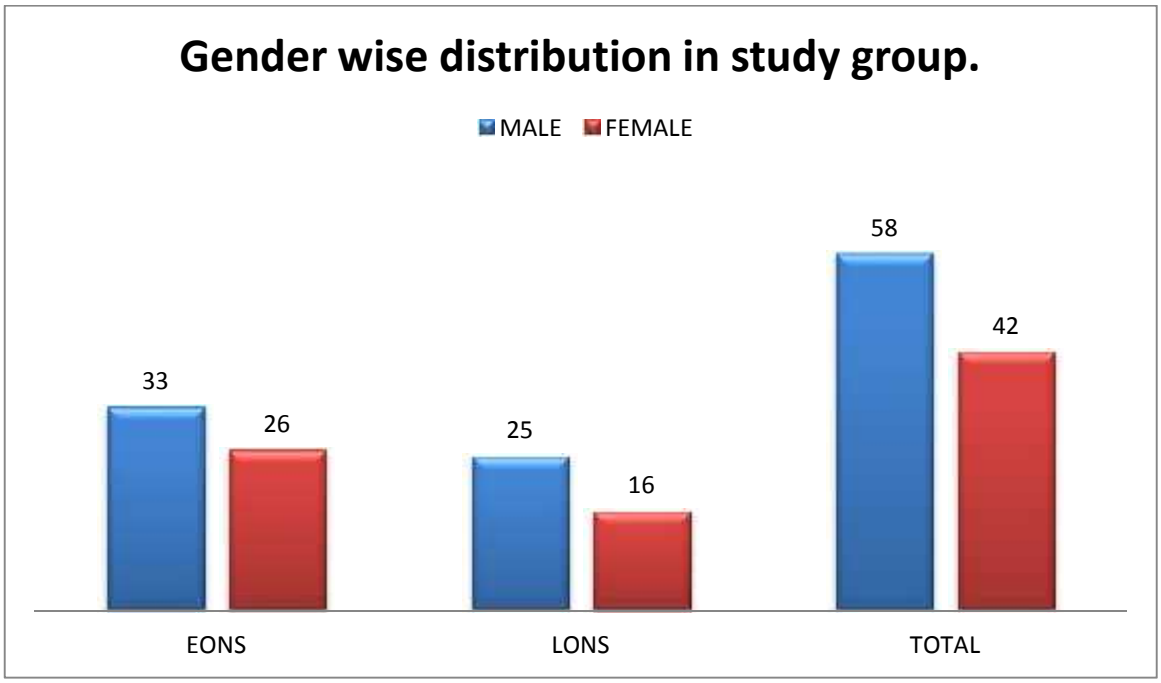
The selected 100 study subjects were analyzed based on age and sex. The

results of the analysis are tabulated in Table 1

Table 1: Gender wise distribution in study group

Age (in days)	**Male**		**Female**		**Total**	
	No.	**%**	**No.**	**%**	**No.**	**%**
EONS (0-3 days)	33	56.86%	26	61.90%	59	59%
LONS (4-28 days)	25	43.10%	16	38.09%	41	41%
Total	**58**	**100%**	**42**	**100%**	**100**	**100%**

FIGURE 1:



Out of the 100 neonates, 59 (59%) were in the age group of 0-3 days and these neonatal sepsis are called as early onset neonatal sepsis (EONS). In EONS, 33 cases are male and 26 female cases.

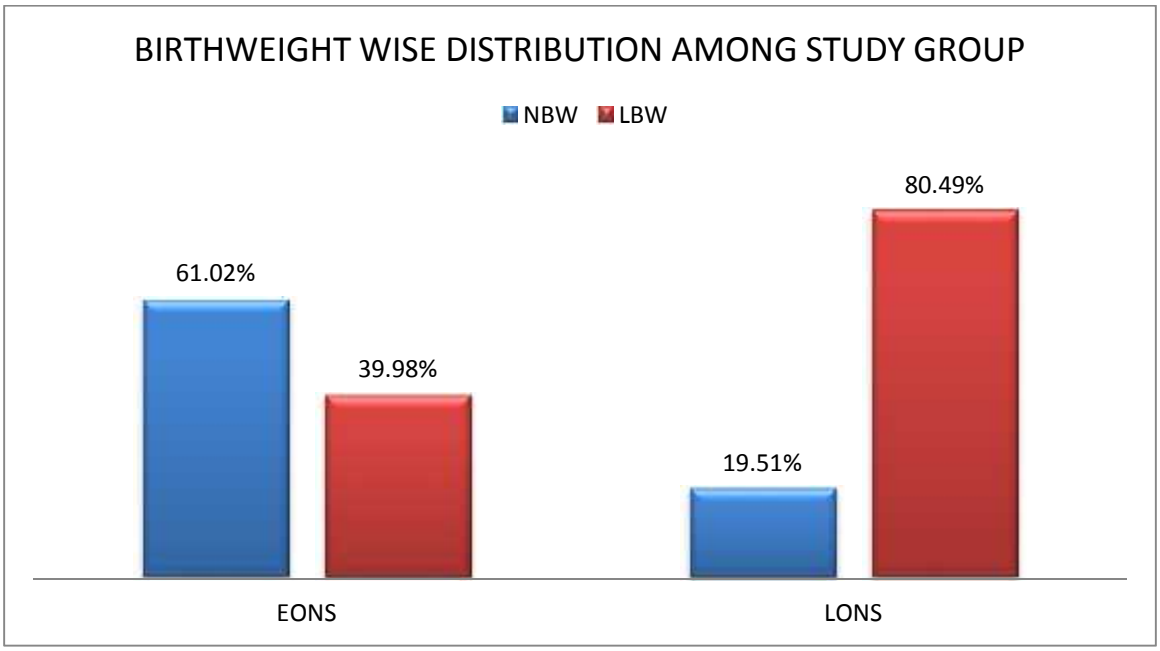
The remaining 41 (41%) were in the age group of 4-28 days and these neonatal sepsis are called late onset neonatal sepsis (LONS). In LONS, 25 cases are male and 16 female infants. (Table 1 and Figure 2).

The chi-square value is 0.2526 and the P-value is 0.6152. It was found to be statistically not significant.

TABLE 2: BIRTHWEIGHT WISE DISTRIBUTION AMONG STUDY GROUP

Birth weight	EONS		LONS		TOTAL	
	No.	%	No.	%	No.	%
Normal Birth weight (≥ 2.5 Kgs)	36	61.02%	8	19.51%	44	44%
Low Birth weight (< 2.5 Kgs)	23	39.98%	33	80.49%	56	56%
Total	59	100%	41	100%	100	100%

FIGURE:2



The table : 2 and figure : 3 shows that out of 100 cases studied , 44% of neonates were of normal birth weight (≥ 2500 gms) and 56% of neonates were of low birth weight (<2500 gms).

Among 59 cases of EONS, 36 (61.02%) cases were normal birth weight and 23 (39.98%) cases were low birth weight.

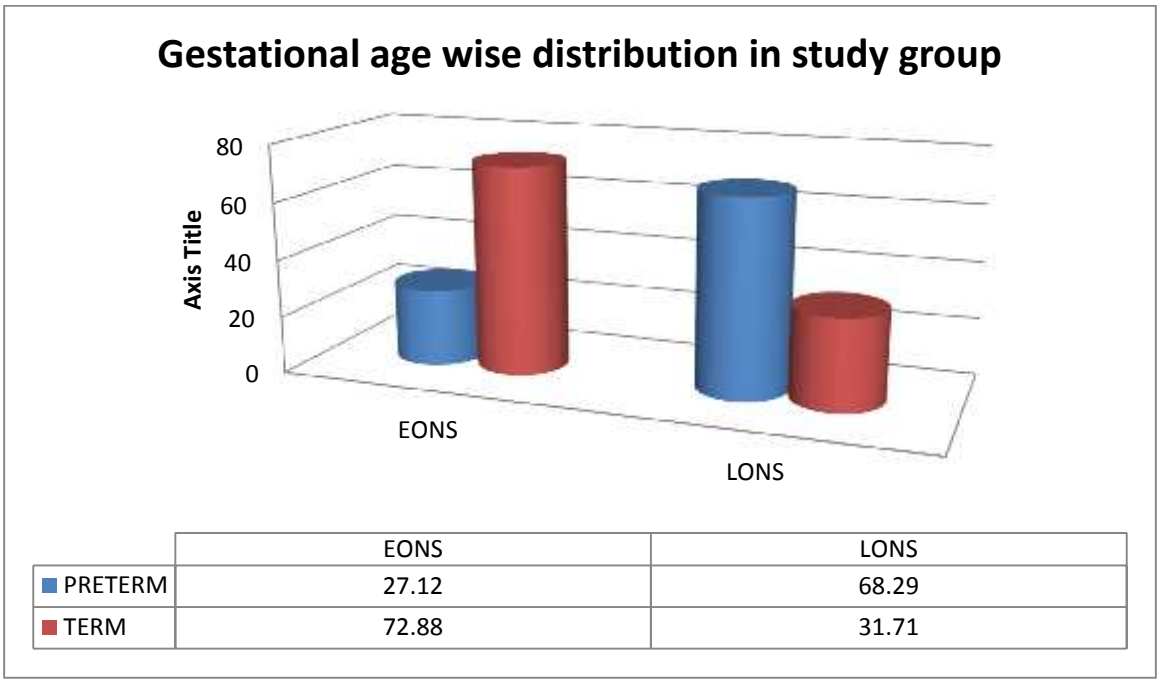
Among 41 cases of LONS, 33 (80.49%) cases were found low birth weight only 8 cases (19.51%) were normal birth weight.

The chi-square value is 16.9118 and the P-value is 0.000039. It was found to be statistically significant.

TABLE:3 Gestational age wise distribution in study group

Gestational age	EONS		LONS		TOTAL	
	No.	%	No.	%	No.	%
Preterm (< 37 weeks)	16	27.12%	28	68.29%	44	44%
Term (completed 37 weeks)	43	72.88%	13	31.71%	56	56%
Total	59	100%	41	100%	100	100%

FIGURE:3



	EONS	LONS
PRETERM	27.12	68.29
TERM	72.88	31.71

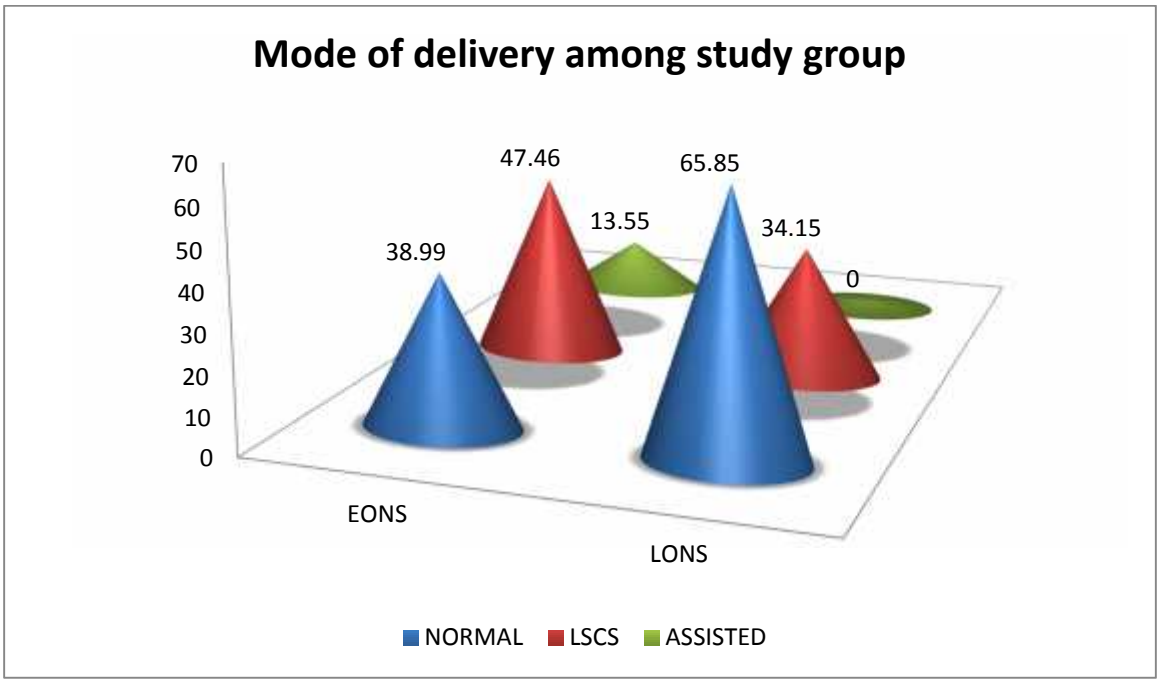
Out of 100 cases studied , 44% of neonates were found to be preterm (< 37 weeks of gestation) and 56% of neonates were found to be term neonates (completed 37 weeks).

Among 59 cases of EONS, 16 (27.12%) cases were found to be preterm and 43 (72.88%) cases were term neonates. Among 41 cases of LONS, 28 (68.29%) cases were found to be preterm and 13 cases (31.71%) were term neonates.(Table : 3 and Figure:4) The chi-square value is 16.6434 and the P-value is 0.000045. It was found to be statistically significant.

TABLE: 4 **Mode of delivery among study group**

Mode of delivery	**EONS**		**LONS**		**TOTAL**	
	No.	**%**	**No.**	**%**	**No.**	**%**
Normal	23	38.99%	27	65.85%	50	50%
Lower segment Caesarean Section	28	47.46%	14	34.15%	42	42%
Assisted	8	13.55%	0	0%	8	8%
Total	59	100%	41	100%	100	100%

FIGURE 4:



Among the 100 neonates studied, 50 (50%) of the neonates were delivered normally, 42 (42%) of neonates were delivered by Lower segment Caesarean Section (LSCS) and only 8 (8%) of neonates were delivered by assisted vaginal deliveries.

Among 59 cases of EONS, 8 (13.55%) neonates were delivered by assisted vaginal delivery, 28 neonates (47.46%) were delivered by LSCS and 23 neonates(38.99%) were delivered by labour natural. Among 41 cases of LONS, 14 neonates (34.15%) were delivered by LSCS and 27 neonates(65.85%) were delivered by labour natural. (Table : 4 and Figure : 5)

The chi-square value is 10.073 and the P-value is 0.006496. It was found to be statistically significant.

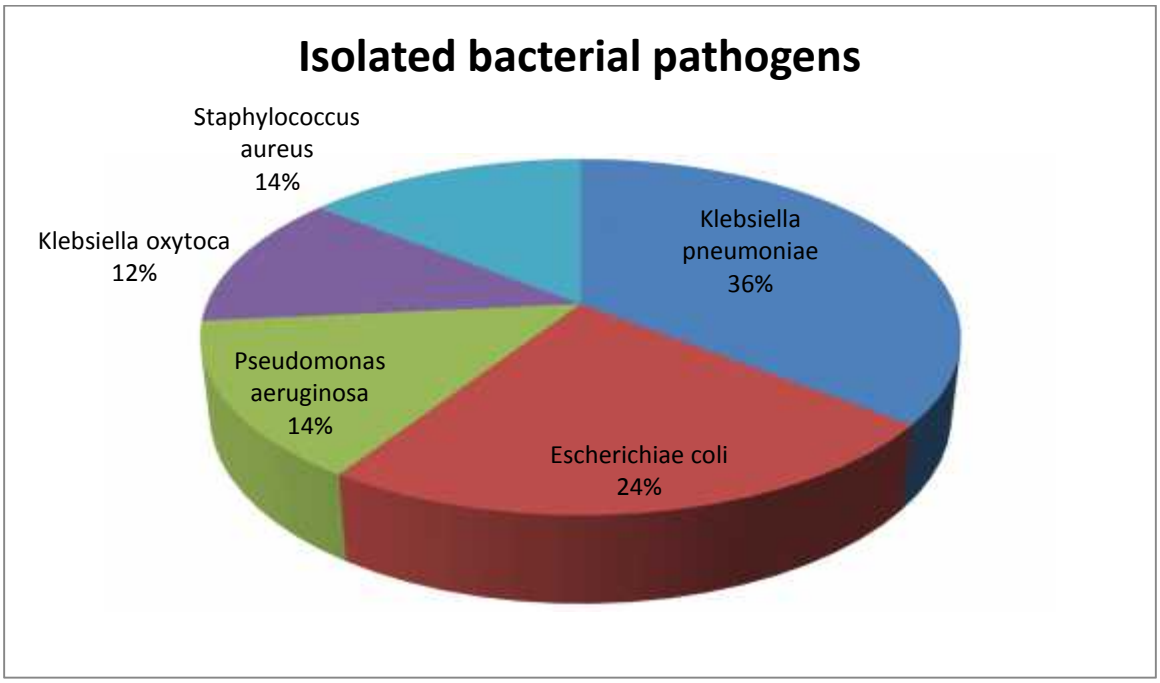
BLOOD CULTURE RESULTS:

Out of the blood samples collected from 100 participants, 26 cases were found to be positive for blood culture. The organisms isolated were *Klebsiellapneumoniae* in 9 neonates*(35%),Escherichiae coli* in 6 neonates (23%), *Pseudomonas aeruginosa* in 4 neonates(14%), *Klebsiellaoxytoca in 3*neoates*(12%)*, and *Staphylococcus aureus in 4* neonates*(15%).Klebsiellaspp*was found to be a most common organism in both early and lateonset sepsis.

TABLE:5 Isolated bacterial pathogens

Bacterial isolate	
Klebsiellapneumoniae	9(36%)
Escherichiae coli	6(24%)
Pseudomonas aeruginosa	4(14%)
Klebsiellaoxytoca	3(12%)
Staphylococcus aureus	4(14%)

FIGURE:5



Antimicrobial sensitivity pattern of the isolated organisms:

Out of 9 *Klebsiellapneumoniae* isolates, 8 were resistant to third generation cephalosporins with Extended Spectrum Beta Lactamase (ESBL) phenotype. It contributes 89 % among of *Klebsiellapneumoniae* isolates. Only one isolate was sensitive to third generation cephalosporin group.

In 3Klebsiella*oxytoca* isolates, two isolates were found to be resistant to third generation cephalosporin with ESBL phenotype and remaining one isolate was sensitive to third generation cephalosporin.

Out of 6 *Escherichiae coli* isolates, five (80%) were sensitive to third generation cephalosporin. Only one case (20%) was resistant to third generation cephalosporin with ESBL phenotype. But all 22 gram negative isolates were sensitive to imipenem.

Among four *Staphylococcus aureus,* 3 isolates (75%) were methicillin resistant strain (MRSA).

Out of 26 bacterial isolates, 21 isolates (81%) were sensitive to amikacin , 20 isolates (76.9%) were sensitive to gentamicin , 21 isolates (81%) were sensitive to ciprofloxacin and 12isolates (46.1%) were sensitive to cotrimoxaxole.

TABLE 6:SENSITIVITY PATTERN OF BACTERIAL ISOLATE:

Antibiotics	*K.pneumoniae*		*K.oxytoca*		*E.coli*		*P.aerugenosa*		*S.aureus*	
	n=9	%	n=3	%	n=6	%	n=4	%	n=4	%
Gentamicin	7	78	2	67	5	83	2	50	4	100
Amikacin	7	78	2	67	5	83	3	75	4	100
Ceftriaxone	2	22	1	33	5	67	1	25	-	-
Ceftazidime	2	22	1	33	5	67	1	25	-	-
Co trimoxazole	5	56	1	33	4	67	0	0	2	50
Ciprofloxacin	7	78	2	67	5	83	3	75	4	100
Imipenem	9	100	3	100	6	100	4	100	-	-
Ampicillin	-	-	-	-	-	-	-	-	2	50
Cefoxitin	-	-	-	-	-	-	-	-	2	50
Erythromycin	-	-	-	-	-	-	-	-	3	75
Clindamycin	-	-	-	-	-	-	-	-	3	75

C-reactive protein results in study group:

Out of 100 clinically suspected neonatal cases, 34 neonates were C-reactive protein test positive. Out of the 34 positive cases 22 neonates (64.71%) were EONS and 12 neonates (35.29%) were of LONS.

FIGURE 6: CRP positive cases in study group

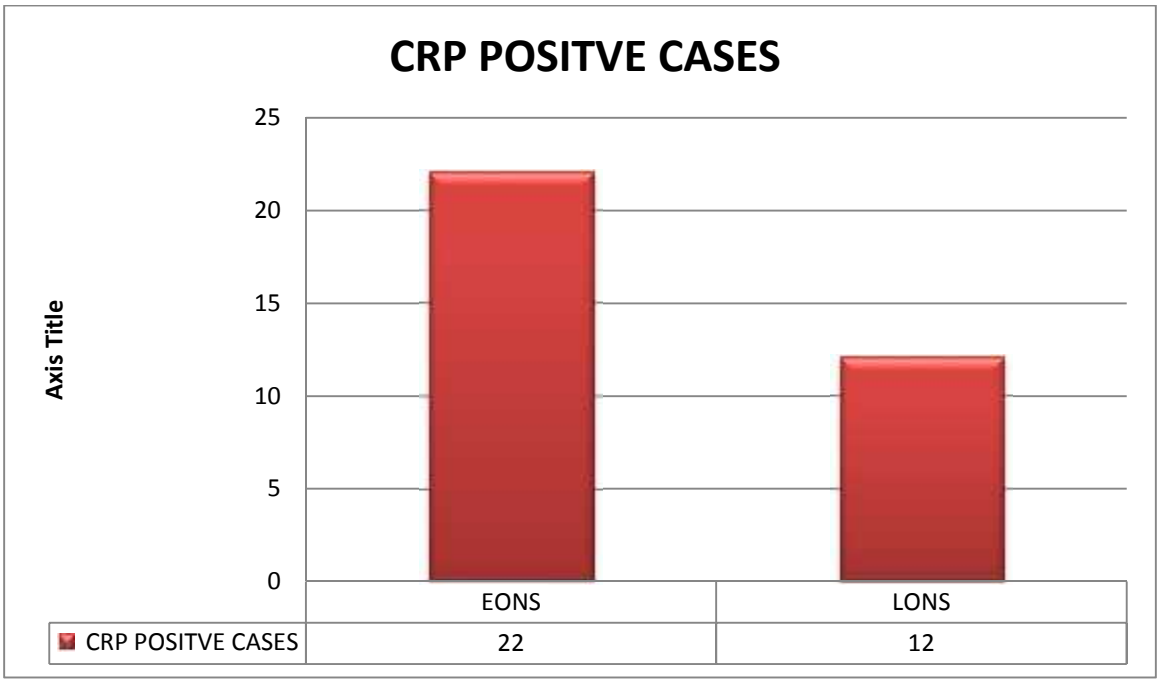


TABLE 7: Association between blood culture and CRP

CRP	Blood Culture		TOTAL
	POSITIVE	Negative	
POSITIVE	14	20	34
NEGATIVE	12	54	66
T0TAL	26	74	100

Detection of CRP by latex agglutination method was evaluated for its sensitivity and specificity against blood culture as reference test.

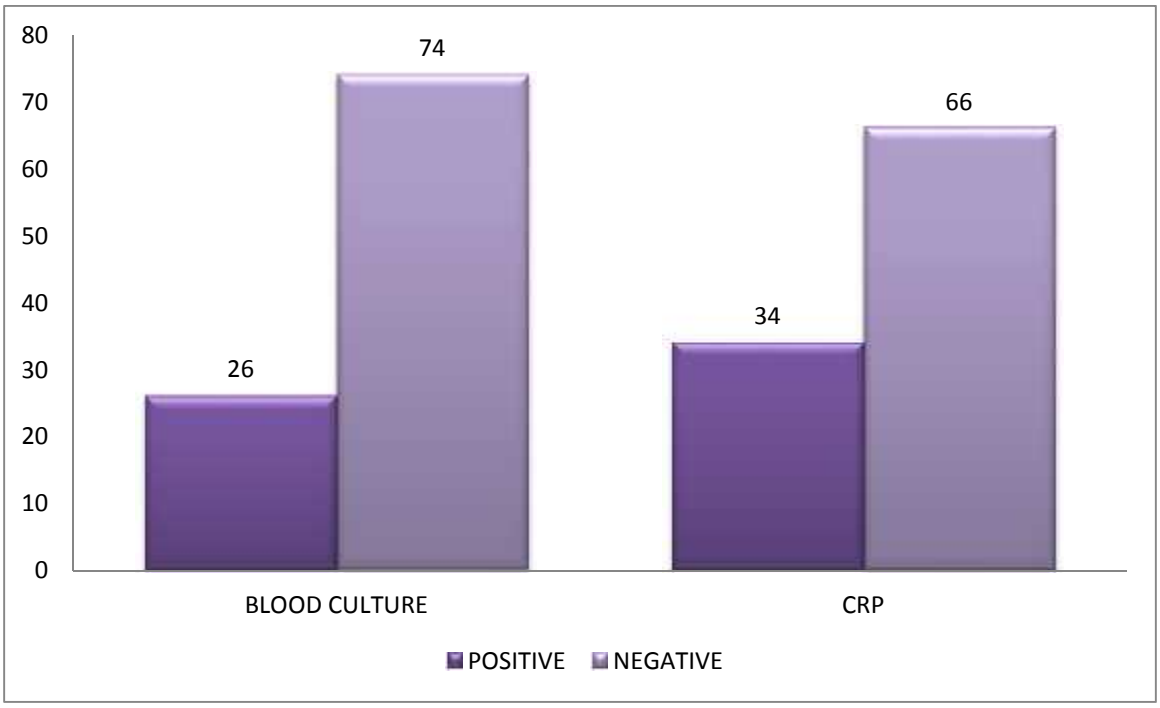
Sensitivity $=\frac{T}{T+F}=\frac{1}{2}\times 100=53.84\%$

Specificity $=\frac{T}{T+F}=\frac{5}{7}\times 100=72.97\%$

Positive predictive value $=\frac{T}{T+F}=\frac{1}{3}\times 100=41.18\%$

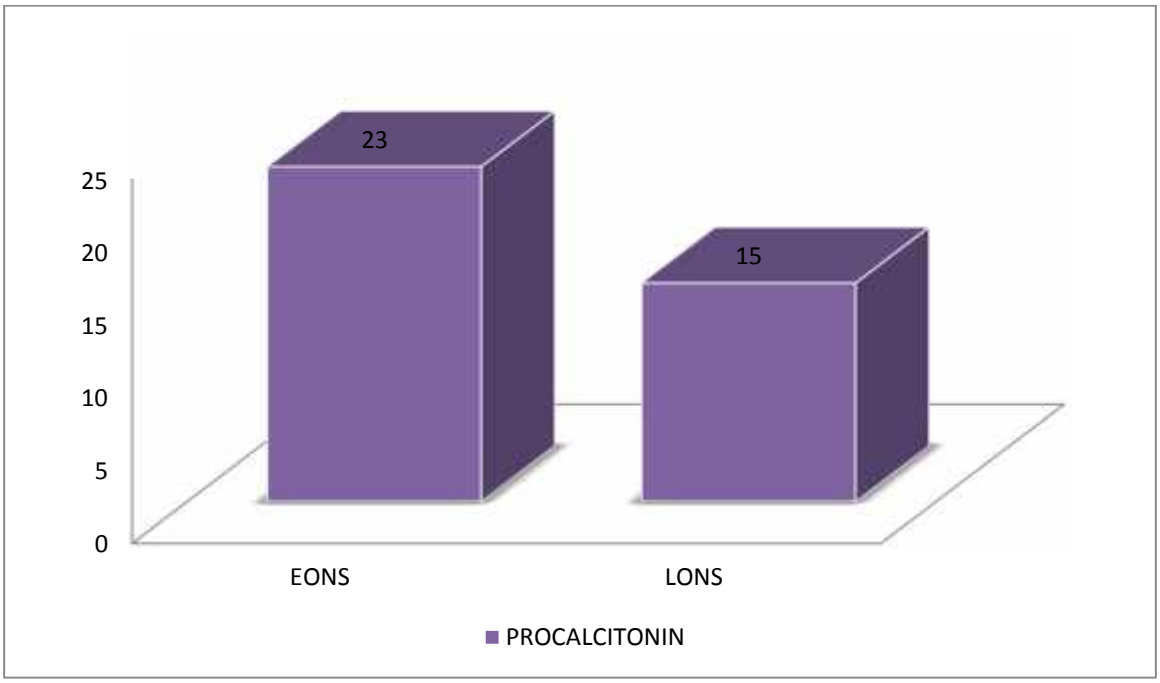
Negative predictive value = $\frac{T}{T+F} = \frac{5}{6} \times 100 = 81.82\%$

FIGURE 7:Association between blood culture and CRP



From the above table :9 and figure : 11, sensitivity of CRP latex agglutination test was 53.84% when evaluated against blood culture, as a reference test.Specificity of CRP latex agglutination test was 72.97% compared to culture and positiveand negative predictive value were 41.18% and 81.82% respectively. It was found to be statistically significant. The chi-square value is 6.167. P value is 0.0130.

FIGURE 8: PROCALCITONIN POSITIVE CASES IN STUDY GROUP



Out of 100 clinically suspected neonatal cases, 38 neonates were Procalcitonin positive. On the 38 positive cases 23 (60.53%) neonates were EONS and 15 (39.47%) cases were of LONS.

TABLE 8: ASSOCIATION OF BLOOD CULTURE AND PROCALCITONIN

PROCALCITONIN	BLOOD CULTURE		TOTAL
	POSITIVE	NEGATIVE	
POSITIVE	25	13	38
NEGATIVE	1	61	62
T0TAL	26	74	100

Detection of serum Procalcitonin level by ELISA method was evaluated for its sensitivity and specificity against blood culture, as reference test.

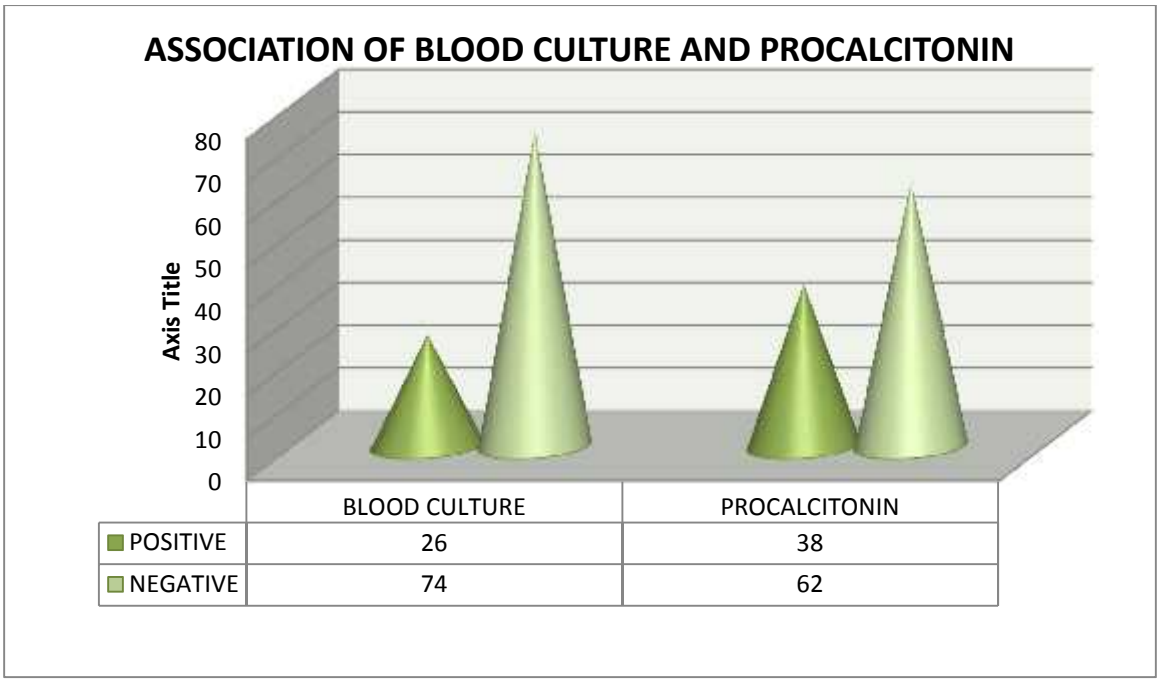
Sensitivity $= \frac{T}{T+F} = \frac{2}{2} \times 100 = 96.15\%$

Specificity $= \frac{T}{T+F} = \frac{6}{7} \times 100 = 82.43\%$

Positive predictive value = $\frac{T}{T+F} = \frac{2}{3} \times 100 = 65.79\%$

Negative predictive value = $\frac{T}{T+F} = \frac{6}{6} \times 100 = 98.39\%$

FIGURE 9:

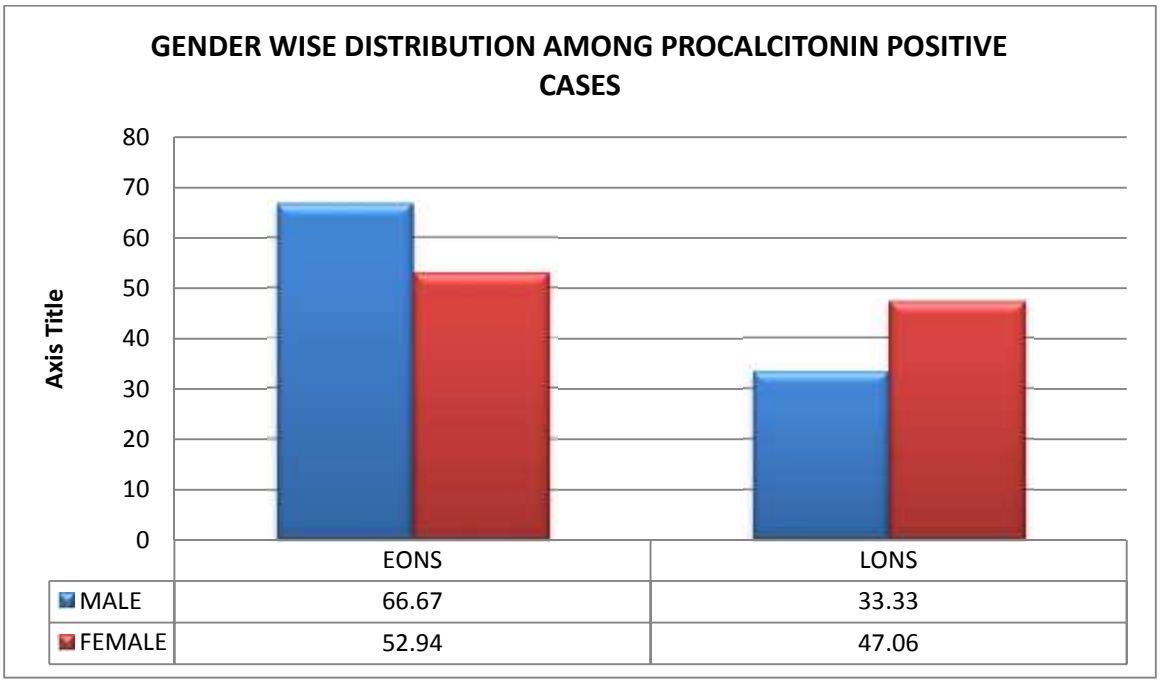


From the above table:10 and figure :13, sensitivity of detection of Procalcitonin by ELISA method was 96.15% when evaluated against culture ,as a reference test. Specificity of this test was 82.43% compared to culture, and positive and negative predictive value were 65.79% and 98.39% respectively. The chi-square value is 50.434. P-value is < 0.00001. It was found to be statistically significant.

TABLE 9: GENDER WISE DISTRIBUTION AMONG PROCALCITONIN POSITIVE CASES:

SEX	EONS		LONS		TOTAL	
	No.	**%**	**No.**	**%**	**No.**	**%**
Male	14	66.67%	7	33.33%	21	100%
Female	9	52.94%	8	47.06%	17	100%

FIGURE 10:

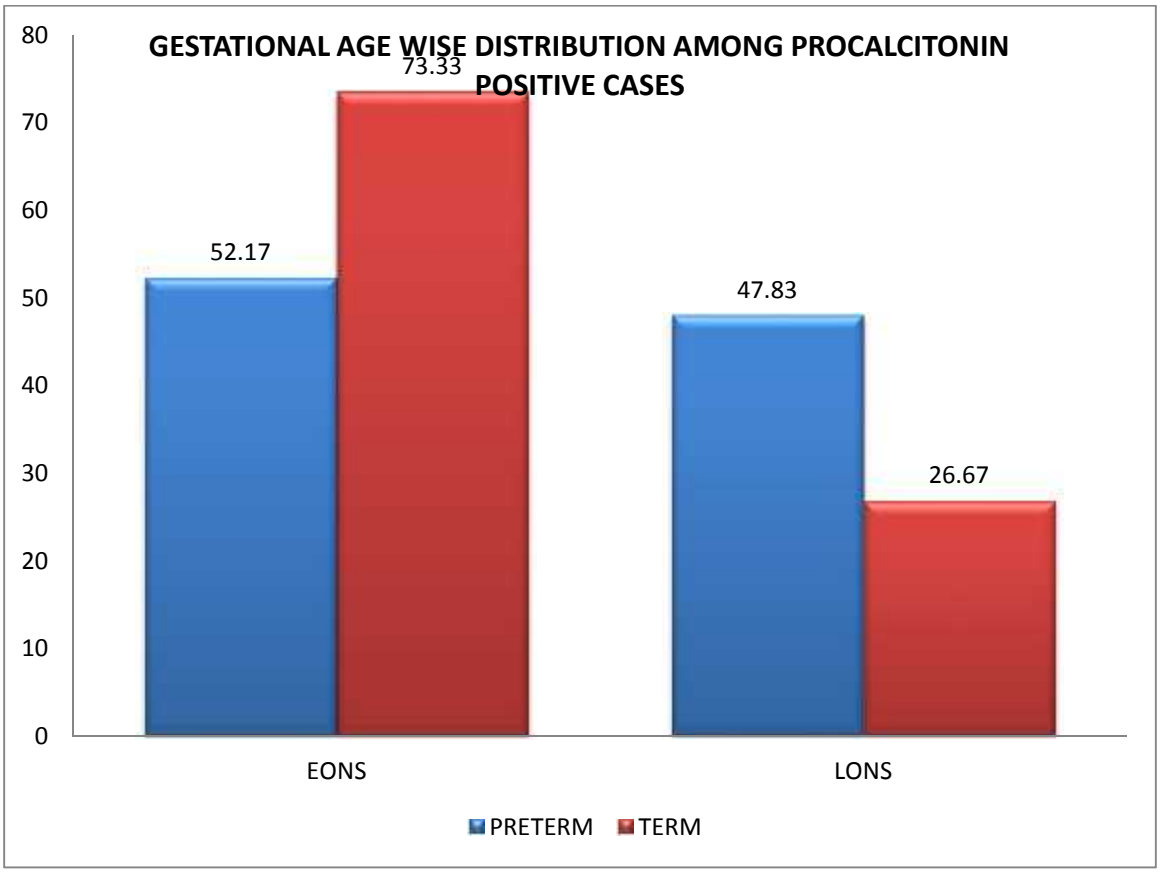


Above figure 10 shows among 38 procalcitonin positive cases, 21 cases were male neonates and 17 cases were female neonates.

TABLE 10: GESTATIONAL AGE WISE DISTRIBUTION AMONG PROCALCITONIN POSITIVE CASES

Gestational age	EONS		LONS		TOTAL	
	No.	%	No.	%	No.	%
Term	11	73.33%	4	26.67%	15	100%
Preterm	12	52.17%	11	47.83%	23	100%

FIGURE 11:

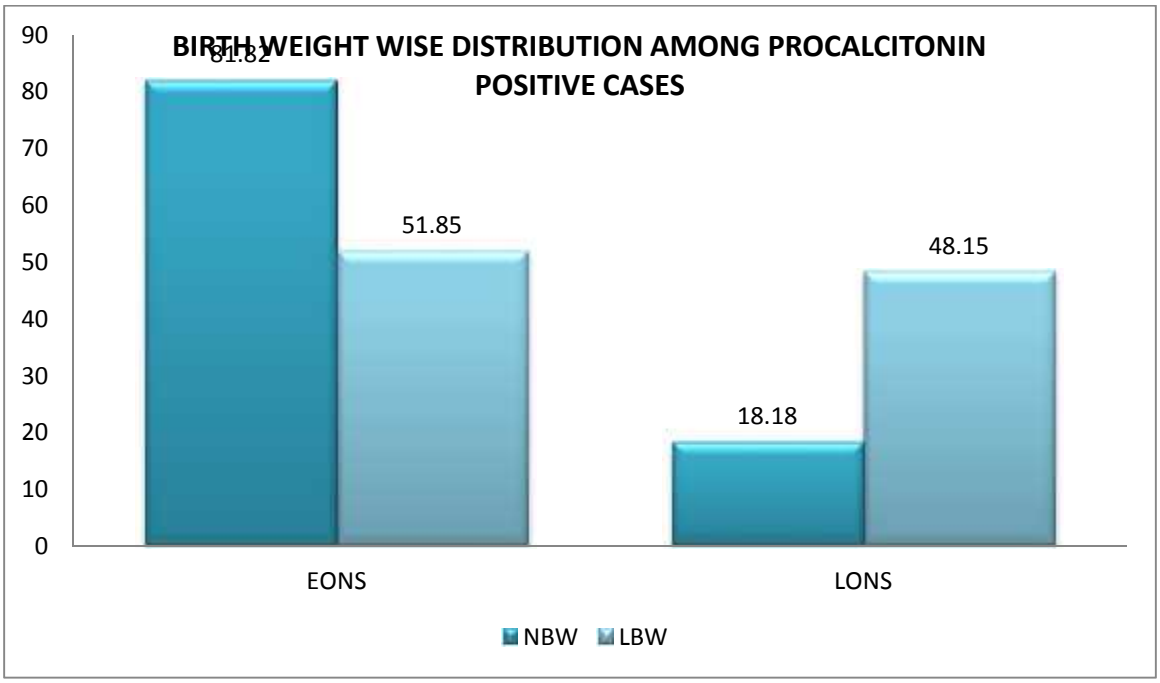


Above figure 11 shows that among 38 procalcitonin positive cases, 15 cases were term neonates and 23 cases were preterm neonates.

TABLE 11: BIRTH WEIGHT WISE DISTRIBUTION AMONG PROCALCITONIN POSITIVE CASES

BIRTH WEIGHT	EONS		LONS		TOTAL	
	No.	%	No.	%	No.	%
NBW	9	81.82%	2	18.18%	11	100%
LBW	14	51.85%	13	48.15%	27	100%

FIGURE 12:



Above figure 12 shows among 38 procalcitonin positive cases, 11 cases were of normal birth weight and 27 cases were of low birth weight neonates.

TABLE12: PROCALCITOIN QUANTITATIVE ASSAY

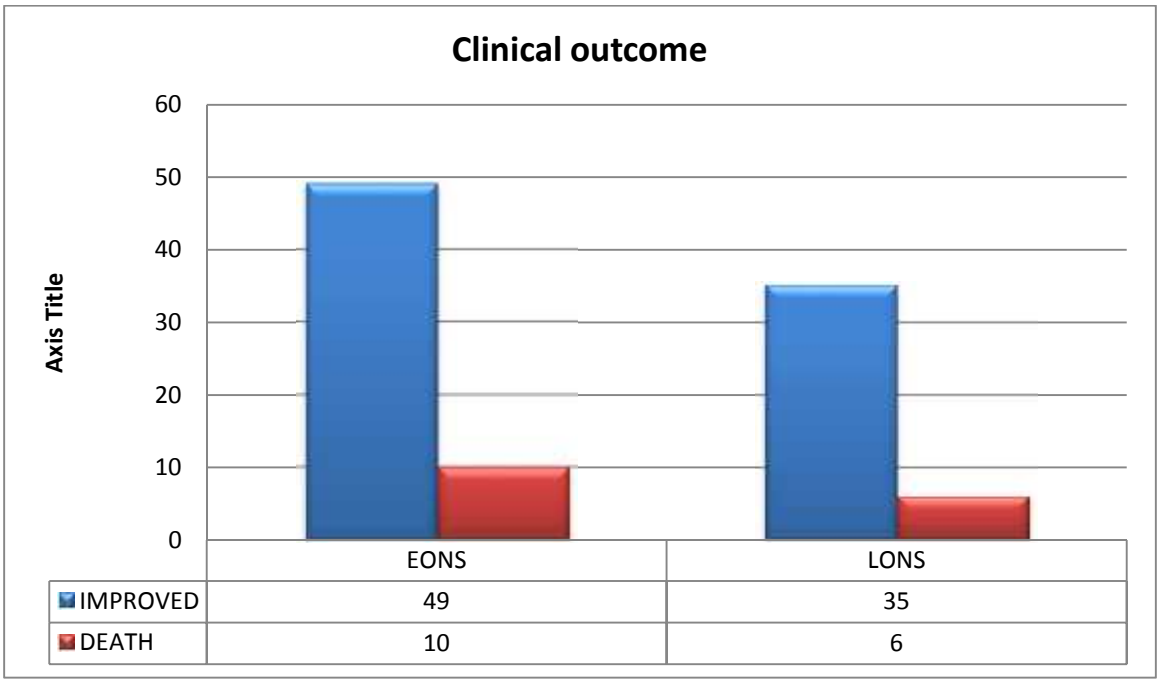
GROUP	NO OF CASES
NEGATIVE($< 0.5n\ /m$)	62
WEAKLY POSITIVE (0.5-2*ng/ml*)	13
STRONGLY POSITIVE ($> 2n\ /m$)	25
TOTAL	100

Out of 38 Procalcitonin positive cases 25 infants were strongly positive with procalcitoni level $> 2n\ /m$ and remaining 13 cases were positive with low level of Procalcitonin.

Table13 :clinical outcome in study group:

Clinical outcome	EONS		LONS		TOTAL	
	No.	**%**	**No.**	**%**	**No.**	**%**
Improved	49	83.05%	35	85.37%	84	84%
Death	10	16.95%	6	14.63%	16	16%
Total	59	100%	41	100%	100	100%

FIGURE 13:



The neonatal mortality rate of present study was 16% (16 cases). Among these, EONS cases were 10 and LONS cases were 6 cases. Out of 10 EONS cases, 6 cases were positive for blood culture and 4 cases were negative for blood culture. Out of 6 LONS cases, 5 cases were positive for blood culture and one case was negative for blood culture.

Table 14: correlation of procalcitonin level and clinical outcome

Outcome	Procalcitonin level			Total
	Negative	Positive	Strongly positive	
Improved	58(69.05%)	9(10.71%)	17(20.24%)	84
Death	4(25%)	4(25%)	8(50%)	16
Total	62	13	25	100

Figure 14:

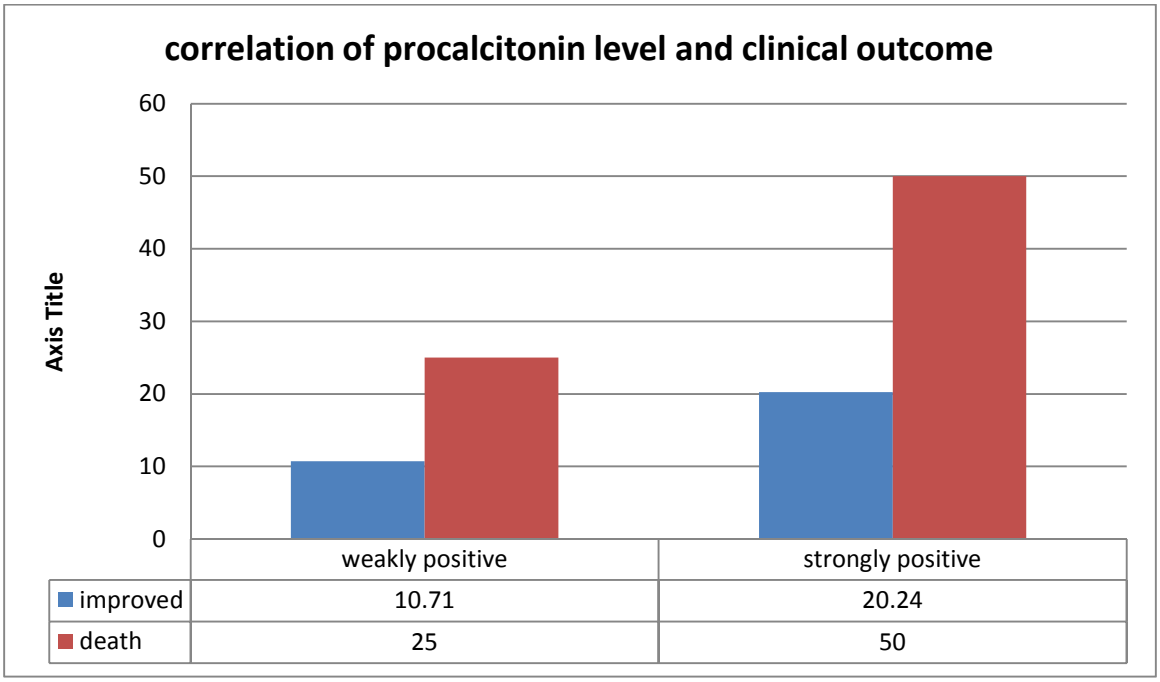


Table 14 shows that 50% of neonatal death were seen in cases with high procalcitonin levels and 25% of death were seen in weakly procalcitonin positive cases.

Table 15: Sensitivity, specificity and predictive values of CRP and PCT.

Test	Sensitivity	Specificity	PPV	NPV	Likelihood ratio	
					Positive	Negative
CRP	53.84%	72.97%	41.18%	81.82%	0.7481	0.7241
PCT	96.15%	82.43%	65.79%	98.39%	1.1808	1.1543

Table 15 shows that Procalcitonin is more sensitive in detecting neonatal sepsis cases than CRP.

6. DISCUSSION:

Neonatal sepsis is defined as a clinical syndrome of bacteremia withsystemic signs and symptoms of infection in the first 4 weeks of life. It encompassesvarious systemic infections of the newborn such as septicemia, meningitis, pneumonia,arthritis, osteomyelitis and urinary tract infections. Superficial infections likeconjunctivitis and oral thrush are not usually included under neonatal sepsis. The aim ofthe present study was to detect thebacteriological profile of neonatal sepsis andantibiotic susceptibility pattern of the isolates and also to determine the value of Creactiveprotein and Procalcitonin in establishing the diagnosis of neonatal sepsis.

6.1 Gender wise distribution in study group:

The present study showed that out of 100 clinically suspected neonatal sepsis cases, increased prevalence was found in male infants than in female infants in the ratio of 1.38 : 1.

Male predominance was also observed in similar studies conducted by YR Khinchi AK *et al* and BambalaPuthattayilZakariya*et al*. This male predominance maybe due to X-linked immune regulatory gene factor contributing to host's susceptibility toinfections in male neonates.

Among 100 clinically suspected neonatal sepsis cases, 59 % of cases were under the age group of 0 – 3 days(<72 hours) and remaining 41 % of cases were under the age group of 4-28 days. FloraChacha*et al* and SucilaThangam*et al* also reported in their study that early onset neonatal sepsis is more prevalent than late onset neonatal sepsis.

In contrast there was a high prevalence of LONS (60%) in a study conducted by NeemaKayange*et al* in Tanzania among 300 clinically suspected neonatal sepsis cases.[6]

6.2 Birth weight wise distribution among study group:

In the current studyamong 59 EONS cases reorted, 61.02% were of normal birth weight and 39.98%were of low birth weight. Among 41 LONS cases reorted, 19.51% were of normal birth weight and 80.49% were of low birth weight.

Similar result had been reported in a comparative study by Rabindra N Misra*et al* which showed that out of 75 culture positive cases, 75% of late onset sepsis occurredin low birth weight babies.

Jun-Ho Wu*et al,*also found that more LONS caseswere present in low birth weight babies. The immuno compromised state oflow birth weight babies may be the cause of increased chance of infection in this group.

6.3 Gestational age wise distribution in study group:

The present study shows that out of 59 EONS cases reported, 16 cases (27.12%) were preterm infants and 43 cases (72.88%) were term infants. Among 41 LONS cases reported, 28 cases (68.29%) were preterm and 13 cases (31.71%) were term infants.

Similar study done by BambalaPuthattayilZakariya*et al* and SubhranshuSekharKar*et al* showed increased prevalence of neonatal sepsis among preterm infants when compared with term neonates.

This increased susceptibility of neonatal sepsis among preterm infants could be attributed to inherent deficiency of both humoral and cellular immunity during the firstweek of life.

6.4 Mode of delivery in the study group:

In the current study out of 59 EONS cases, 23 cases(38.99%) were delivered by labour natural, 28 cases(47.46%) were by LSCS and 8 cases(13.55%) were delivered by assisted delivery. Among 41 cases of LONS, 14 neonates (34.15%) were delivered by LSCS and 27 neonates(65.85%) were delivered by labour natural.

This finding was comparable with that of the study which was conducted by Tuuli and Odibo andAfsharpaiman et al,. They demonstrated that cesarean section could be associated with several adverseneonatal events, including respiratory

complications. This leads to higher rates of NICU admission and higherchances of developing newborn sepsis.

6.5Bacterial isolates in blood culture:

The current study shows that among 100 suspected sepsis cases, blood culture waspositive in 26 cases (26%).

Similar positivity percentage of blood culture had been reported in the study bySucilaThangam*et al* during April – September 2010 which revealed 28% of positiveblood culture in 50 samples.

Shrestha R K *et al* in Nepal medicalcollege, Kathmandu, during the period of July 2011 to January 2012 reported similarrange results of 30.8% of blood culture positive cases.

In contrast there was a high positivity report of blood culture in the study done byRajalakshmiVishwanathan*et al* in Kolkatta during March 2009 –August 2010 which revealed 46.3% of blood culture positivity in 216 samples. In contrast low positivity ofblood culture had been reported in studies by SubhranshuSekarKar*et al* inBhubaneshwar during the study period (2007–2010) which revealed 16.2% of blood culture positivity in 120 samples.

Reasons for comparative variability in blood culture positivity of various studies might be due to administration of prior antibiotics from primary centre, infection withanaerobes and effective control in spread of hospital acquired infection.

Another reason attributed to the variable positive result may depend on the mode of test employed for culture. Commonly used conventional test is time consuming and itsyield is of significantly low sensitivity when compared to fully automated system whichis less time consuming and has the benefit of continuous monitoring with highsensitivity.

Out of the 26 isolates, 22 were gram negative bacteria and only 4 isolates were gram positive bacteria. Among gram negative organisms *Klebsiellapneumoniae*was found to be the mostcommon organism in both early and late onset sepsis.

Similar results were observed in studies done by BambalaPuthattayilZakariya et al ,NeemaKayange*et al*and Iregu KC *et al*.

*Klebsiellapneumonia*e is one of most common pathogen profoundly found in neonatal intensive care ward environment and various equipment in neonatal ward. This could be the cause for increased incidence of *Klebsiellapneumoniae*as a causative organism for neonatal sepsis.

In contrast, in a study conducted by Shrestha R K *et al* in Nepal medical college, Kathmandu, during the period of July 2011 to January 2012, *Staphylococcus aureus*(56.8%) had been reported as main etiological agent in neonatal sepsis and followed by*Klebsiellapneumoniae*(21.7%), *Pseudomonas aeruginosa* (13.4%) and others

6.6 Antibiotic susceptibility pattern of isolated pathogens:

The present study showed that among 12 *Klebsiellaspp*, 9 isolates were *Klebsiellapneumoniae*species and remaining 3 isolates were *Klebsiellaoxytoca*.

Out of 12 isolates of *Klebsiellaspp*, 83% (10 isolates) were resistant to third generation cephalosporin with ESBL phenotype.

A similar study done by Iregu KC *et al* found that 87% of *Klebsiella pneumoniae*isolates were found to be ESBL producers. Another similar study done in North India by Kaistha N *et al* found that 88% gram negative isolates were resistant tothird generation cephalosporin.

But this was contrary to the observations of BambalaPuthattayilZakariya*et al* in Jawaharlal NehruInstitute of Post graduate, Medical Education and Research Puducherry, in which 32% of *Klebsiellapneumoniae*isolates were found to be ESBL producers.

The thirdgeneration cephalosporin are commonly used empirically in all cases of clinicallysuspected sepsis in present study centre. It could be the main cause for increasedincidence of ESBL in the present study.

But all 12 isolates of *Klebsiellaspp*were sensitive to imipenem. Similar result wasobtained in the study done by NeemaKayange*et al* with about 95% of *Klebsiella spp* sensitive to imipenem.

The present study shows that among four *Pseudomonas aeruginosa*, 2 isolates (50%) were resistant to gentamicin, 3 isolates (75%) were resistant to third generationcephalosporin and one isolate was resistant to ciprofloxacin. But all four isolates were sensitive to imipenem and Piperacillin-Tazobactum. This finding similar to study of BambalaPuthattayilZakariya*et al*.

The current study shows that among four *Staphylococcus aureus*, 3 isolates (75%)were resistant to methicillin (MRSA), which is comparable with Ramesh Bhat Y*et al* study results.

Increased prevalence of MRSA may be due to healthy carriers in hospital staffs and relatives.

6.7 Comparison of C-reactive protein with blood culture:

The current study showed that out of 100 clinically suspected sepsis cases 34 were positive for CRP. Out of the 34 positive cases 22 neonates (64.71%) were EONS and 12 neonates (35.29%) were of LONS**.** The sensitivity of CRP for proven sepsis (more than 6 µg/ mL)was 53.84%, its specificity was 72.95%, its positive predictive value was 41.18% and its negative predictive value was 81.82%.

Similar observation was reported in study done by SucilaThangam*et al*, that sensitivity of CRP were 50%, its specificity was 69.4%, its positivepredictive value was 38.8% and its negative predictive value was 78.1%.

Contrary results were observed in a study done by Doeller H *et al*, reported that CRP was 63% sensitive, 97% specific, its positive predictive value was 83% and negative predictivevalue was 91%.

A study done by Franz A R *et al* found that 28% of CRP sensitivity, 97% of specificity, 81% of positive predictive value and 77% of negative predictive value. This study showed lower sensitivity compared to the present study.

6.8 Comparison of Procalcitonin with blood culture:

In the current study, totally 38 neonates were Procalcitonin positive. Among them 23 (60.53%) neonates were EONS and 15 (39.47%)cases were of LONS. The sensitivity of procalcitonin for proven sepsis was 96.15%, itsspecificity was 82.43%, its positive predictive valve was 65.79% and its negative predictive value was 98.39%.

In a similar study by Sakha *et al*. showed that the sensitivity, specificity, positive predictive valueand the negative predictive value of PCTwere 66.7%, 50%, 28.6%and 83.3% which is lower than that of the current study.

Chiesa *et al.,* studied the reliability of the PCT concentration in 28 infants who had a severe early onset of neonatal sepsis. They found that the sensitivity, specificity, PPV and NPV were 92.6, 97.5, 94.3 and 96.8%, respectively. In this study the specificity is higher than that of current study.

Vazzalwar et al., assessed PCT for the diagnosis of neonatal sepsis in 67 neonates was found that the sensitivity and specificity were of 97% and 80% respectively.

6.9 Gender wise distribution among procalcitonin positive cases:

The current study shows among 38 Procalcitonin positive cases, male neonates were of 55.26% and female neonates were of 44.74%. Similar studies by RekhaSriram*et al,* Kuruvilla*et al* and Shrestha *et al* also showed that male neonates werepredominance in culture positive cases. This male predominance maybe due to X-linked immune regulatory gene factor contributing to host's susceptibility toinfections in male neonates

6.10 Gestational age wise distribution in procalcitoin positive cases:

The present study shows that out of 38procalcitonin positive cases, 23 cases (60.53%) were preterm babies and remaining 15 cases (39.47) were term babies.

In similar study done by Boma*et al,* BambalaPuthattayilZakariya*et al,* Shrestha *et al* and Kuruvilla*et al* observed increasedprevalence of neonatal sepsis among preterm neonates when compared with termneonates. The immuno compromised state of low birth weight and preterm babies may bethe cause for more infection in these group

6.11Birth weight wise distribution in Procalcitonin positive cases:

In the current study out of 38 Procalcitonin positive cases, 27 (71.05%) cases were low birth weight (less than 2500 gm) neonates and 11 cases(28.95%) were of normal birth (weight above 2500 gms)

In similar study by NeemaKayange*et al* , Rabindra N Misra*et al* and SubhranshuSekharKar*et al*., it was found that increased prevalence of neonatal sepsis among low birthweight neonates when compared with normal birth weight neonates.

6.12 Clinical outcome and procalcitonin levels:

The neonatal mortality rate of present study was 16% (16 cases). Among these, EONS cases were 10 and LONS cases were 6 cases.

Current study showed that higher mortality rate was present in neonates with higherProcalcitonin level (more than 2ng/ml). Similarly, Brunkhorst et al have even demonstrated that very high PCT values were related to septic shock and death. Jensen JU *et al* also demonstrated that patients with septic process present lessmortality risk with PCT levels less than 1mg/dl and had more mortality risk with PCT value more than 1mg/dl.

In the current study, the PCT levels were remarkable high in neonateswith proven sepsis and also in suspected sepsis cases.It is comparable withthe study conducted

by YadollaZahedpasha et al., andMonneretet al,. In the present study PCT detected nearly all blood culture positive cases.

In a study by Carolet al., showed that procalcitonin is moresensitive than the CRP in the diagnosis of septicemia, meningitis andurinary tract infection. In our study proclacitonin was positive in almost all culture positive cases when compared to CRP. PCT also detects more culture negative clinically suspected sepsis cases than CRP. These findingssupport the usefulness of the PCT to establish an early diagnosis of neonatalsepsis.

The current study confirmed the findings of various authors that Procalcitonin was more sensitive than CRP in detection of neonatal sepsis. In neonatal sepsis, serum Procalcitonin level rise earlier than the CRP level.So serum Procalcitonin level detection is mainly useful in earlydetection of neonatal sepsis. Detection of serum Procalcitonin level also detects the severity ofinfection and evaluation of the response to antibiotic treatment.

Early diagnosis of neonatal sepsis by Procalcitonin evaluation helps to prevent neonatalmortality and morbidity and avoid unnecessary use of empirical antibiotics whichin turn helps in preventing drug resistance.

7. SUMMARY

The present study aimed at detecting the bacteriological profile in neonatal sepsis and antibiotic susceptibility pattern of isolates in 100 clinically suspected cases and alsodetect the role of CRP and Procalcitonin in the diagnosis of neonatal sepsis.

- Out of 100 clinically suspected neonatal sepsis cases, 58% were male babies and 42% were female babies. Male and Female ratio was 1.38 : 1.
- Out of 100 cases, Early Onset Neonatal Sepsis was 59% and Late Onset Neonatalsepsis was 41%.
- Out of 100 study cases, 26% cases were blood culture positive.
- Among 26 clinical isolates there was increased incidence of gram negative Organisms in which *Klebsiella pneumoniae* (36%) was the most common followed by, *Escherichia coli*(6%), *Pseudomonas aeruginosa*(14%) and *Klebsiella oxytoca*(12%).
- Among 18 *Enterobacteriaceae* isolated , *72%*were resistant to third generation cephalosporin with ESBL phenotype.
- Among the four *Staphylococcus aureus,* 3 isolates (75%) were resistant to methicillin(MRSA).
- Serum CRP level detected by latex agglutination method had 53.84% of sensitivity, 72.97% of specificity, 41.18% of Positive predictive value and 81.82% of negative predictive value against blood culture as a reference test.

J Serum PCT level detected by ELISA method had 96.15% of sensitivity, 82.43% of specificity, 65.79% of Positive predictive value and 98.39% of negative predictive value against blood culture as a reference test.

8. CONCLUSION:

Early recognition and diagnosis of neonatal sepsis are difficult due to the variable and nonspecific clinical presentation. It is important to make an early diagnosis of sepsis, because prompt initiation of antimicrobial therapy improves outcomes**.**

The findings of the present study confirm that the serum level of Procalcitonin is a more reliable marker than the serum levels of CRP. Serum procalcitonin level was superior to serum CRP level in terms of early diagnosis of neonatal sepsis and in detecting the severity of the illness.

The benefit of measuring serum PCT routinely in the diagnosis and follow-up of neonatal sepsis, is that it reduces the hospital costs. Such a benefit might support a wider acceptance of the test in the routine practice.

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ANNEXURE -1

Role of C-reactive protein and Procalcitonin in the diagnosis of neonatal sepsis

PROFORMA

Name : B/O Mrs. : IP No.:

Age : days

Sex :

Gestational a in weeks Preterm / Term

Nature of Delivery : Normal/Assisted/LSCS

Birth weight :

Clinical diagnosis :

Investigation

Lab. No. :

Blood culture Result :

Antibiotics Sensitive to :

Antibiotics Resistance to :

CRP Result :

IL-6 Result : concentration:

Outcome : Recovery and discharge/ death

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CERTIFICATE OF REGISTRATION & APPROVAL OF THE TIREC REF NO:849/MICRO/2016

PROTOCOL TITLE: DIAGNOSTIC ROLE OF PROCALCITONIN IN NEONATAL SEPSIS IN TERTIARY CARE HOSPITAL.

PRINCIPAL INVESTIGATOR: Dr.D. JEYAGANGULI, MBBS.,

DESIGNATION OF PRINCIPAL INVESTIGATOR: POST GRADUATE IN MICROBIOLOGY

DEPARTMENT & INSTITUTION: TIRUNELVELI MEDICAL COLLEGE, TIRUNELVELI

Dear , Dr. D. Jeyaganguli, MBBS., The Tirunelveli Medical College Institutional Ethics Committee (TIREC) reviewed and discussed your application during the IEC meeting held on 05.08.2016.

THE FOLLOWING DOCUMENTS WERE REVIEWED AND APPROVED

1.	TIREC Application Form	☐
2.	Study Protocol	☐
3.	Department Research Committee Approval	☐
4.	Patient Information Document and Consent Form in English and Vernacular Language	☐
5.	Investigator's Brochure	☐
6.	Proposed Methods for Patient Accrual Proposed	☐
7.	Curriculum Vitae of the Principal Investigator	☐
8.	Insurance /Compensation Policy	☐
9.	Investigator's Agreement with Sponsor	☐
10.	Investigator's Undertaking	☐
11.	DCGI/DGFT approval	☐
12.	Clinical Trial Agreement (CTA)	☐
13.	Memorandum of Understanding (MOU)/Material Transfer Agreement (MTA)	☐
14.	Clinical Trials Registry-India (CTRI) Registration	☐

THE PROTOCOL IS APPROVED IN ITS PRESENTED FORM ON THE FOLLOWING CONDITIONS

1. The approval is valid for a period of 2 year/s or duration of project whichever is later
2. The date of commencement of study should be informed
3. A written request should be submitted 3weeks before for renewal / extension of the validity
4. An annual status report should be submitted.
5. The TIREC will monitor the study
6. At the time of PI's retirement/leaving the institute, the study responsibility should be transferred to a person cleared by HOD
7. The PI should report to TIREC within 7 days of the occurrence of the SAE. If the SAE is Death, the Bioethics Cell should receive the SAE reporting form within 24 hours of the occurrence.
8. In the events of any protocol amendments, TIREC must be informed and the amendments should be highlighted in clear terms as follows:
   a. The exact alteration/amendment should be specified and indicated where the amendment occurred in the original project. (Page no. Clause no. etc.)
   b. The PI must comment how proposed amendment will affect the ongoing trial. Alteration in the budgetary status, staff requirement should be clearly indicated and the revised budget form should be submitted.
   c. If the amendments require a change in the consent form, the copy of revised Consent Form should be submitted to Ethics Committee for approval. If the amendment demands a re-look at the toxicity or side effects to patients, the same should be documented.
   d. If there are any amendments in the trial design, these must be incorporated in the protocol, and other study documents. These revised documents should be submitted for approval of the IEC, only then can they be implemented.
   e. Approval for amendment changes must be obtained prior to implementation of changes
   f. The amendment is unlikely to be approved by the IEC unless all the above information is provided.
   g. Any deviation/violation/waiver in the protocol must be informed.

STANDS APPROVED UNDER SEAL

Dr.K.Shantaraman MD
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State of Tamilnadu, South India

MASTER CHART

S.No	Age (in days)	Sex	Gestational Age (in weeks)	Mode of delivery	Birth weight (gms)	Blood Culture	Bacterial isolates	Gentamicin	Amikacin	Ceftriaxone	Ceftazidime	Ceftazidime and Clav	Cotrimoxazole	ciprofloxacin	Imipenem	Ampicillin	Cefoxitin	Erythromycin	Clindamycin	CRP	Procalcitonin	Out come
1	1	Female	Preterm	Normal	2100	N														N	P	Cured
2	3	Male	Preterm	LSCS	1950	N														P	N	Cured
3	3	Male	Term	Normal	1900	N														N	N	Cured
4	1	Female	Preterm	LSCS	2000	P	K.pneumoniae	S	S	S	S	S	S	S	S					P	P	Cured
5	4	Female	Term	LSCS	3200	N														N	N	Cured
6	2	Female	Term	LSCS	2800	P	S.aureus	S	S				S	S		S	S	S	S	P	P	Death
7	4	Male	Term	Normal	3000	N														P	P	Cured
8	3	Male	Term	LSCS	1900	N														N	N	Cured
9	2	Female	Term	LSCS	3100	N														N	N	Cured
10	5	Female	Preterm	Normal	1900	N														P	N	Cured
11	4	Male	Term	LSCS	1600	N														P	N	Cured
12	6	Female	Preterm	LSCS	1700	N														N	N	Cured
13	2	Male	Term	Assisted	3800	N														N	N	Cured
14	2	Male	Term	Normal	1800	P	K.pneumoniae	S	S	R	R	S	R	S	S					P	P	Death
15	4	Female	Term	Normal	3400	N														P	P	Cured

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16	2	Female	Term	LSCS	3900	N														N	N	Cured
17	1	Male	Preterm	Normal	1700	P	E.coli	R	R	R	R	S	R	R	S					P	P	Death
18	4	Male	Term	LSCS	1700	N														P	N	Cured
19	4	Male	Preterm	LSCS	1600	N														N	N	Cured
20	3	Male	Term	Normal	2600	N														N	N	Cured
21	3	Female	Preterm	Normal	2200	N														N	P	Cured
22	2	Male	Term	LSCS	3500	N														P	N	Cured
23	3	Male	Term	Assisted	3500	N														P	P	Death
24	2	Male	Term	Assisted	3300	N														N	N	Cured
25	1	Female	Term	LSCS	2900	N														N	N	Cured
26	2	Female	Preterm	Normal	2100	N														N	N	Death
27	3	Male	Term	LSCS	2400	N														N	N	Cured
28	2	Female	Term	Normal	2400	N														N	N	Cured
29	8	Male	Preterm	Normal	2200	N														N	N	Cured
30	5	Male	Preterm	Normal	2000	N														N	N	Cured
31	6	Female	Term	LSCS	2400	P	P.aeruginosa	R	R	R	R	S	R	R	S					P	P	Death
32	2	Male	Preterm	LSCS	2600	P	K.pneumoniae	S	S	R	R	S	S	S	S					P	P	Death
33	7	Male	Term	LSCS	2100	P	K.pneumoniae	S	S	R	R	S	S	S	S					P	P	Death

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34	6	Male	Term	Normal	2600	N														N	N	Cured
35	3	Male	Preterm	Normal	1900	P	E.coli	S	S	S	S	S	S	S	S					P	P	Cured
36	3	Female	Preterm	Normal	2500	N														N	N	Cured
37	2	Male	Term	LSCS	2600	N														N	N	Cured
38	3	Female	Term	LSCS	2700	N														P	P	Cured
39	3	Male	Term	LSCS	2650	N														N	N	Cured
40	2	Male	Term	Assisted	2700	N														N	N	Cured
41	3	Male	Term	Assisted	2900	P	S.aureus	S	S				S	S		S	S	S	S	N	P	Cured
42	5	Male	Term	Normal	2400	N														P	N	Cured
43	4	Female	Preterm	Normal	2200	N														N	N	Cured
44	3	Male	Preterm	Normal	2000	P	P.aeruginosa	R	S	R	R	S	R	S	S					N	P	Death
45	7	Male	Term	LSCS	3500	N														N	N	Cured
46	1	Male	Term	Assisted	3400	N														P	P	Cured
47	8	Female	Preterm	Normal	1800	P	K.pneumoniae	S	S	R	R	S	R	S	S					P	P	Death
48	3	Male	Term	LSCS	3000	N														N	N	Cured
49	4	Female	Term	Normal	2300	N														N	N	Cured
50	3	Female	Term	Normal	2000	N														P	N	Death
51	2	Male	Term	LSCS	2800	N														N	N	Cured

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52	22	Female	Preterm	LSCS	1700	P	K.oxytoca	S	S	S	S	S	S	S	S					N	P	Death
53	1	Male	Term	Assisted	3300	P	S.aureus	S	S				R	S		R	R	S	S	N	N	Death
54	12	Female	Preterm	LSCS	2800	N														N	N	Cured
55	1	Male	Term	LSCS	3200	N														P	N	Cured
56	3	Male	Preterm	Normal	2400	P	K.pneumoniae	S	S	R	R	S	R	R	R					P	P	Cured
57	24	Female	Preterm	Normal	1900	P	K.pneumoniae	R	R	R	R	S	R	R	S					P	P	Death
58	13	Male	Preterm	Normal	2200	N														P	N	Cured
59	3	Female	Term	LSCS	3200	N														N	N	Cured
60	3	Female	Preterm	Normal	2100	P	E.coli	S	S	S	S	S	S	S	S					P	P	Cured
61	6	Female	Preterm	Normal	3200	N														N	N	Cured
62	6	Male	Term	LSCS	2400	N														N	N	Cured
63	3	Male	Term	LSCS	2700	N														N	N	Cured
64	4	Male	Preterm	Normal	2300	N														N	N	Cured
65	7	Male	Preterm	Normal	2100	P	S.aureus	S	S				R	S		R	R	R	R	N	P	Cured
66	2	Male	Term	LSCS	2800	N														P	P	Cured
67	4	Female	Preterm	Normal	2200	P	P.aeruginosa	S	S	R	R	S	R	S	S					N	P	Cured
68	3	Female	Term	LSCS	2700	N														N	N	Cured
69	4	Male	Preterm	Normal	2300	N														N	N	Death

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70	3	Male	Preterm	Normal	2100	P	K.pneumoniae	R	R	R	R	S	S	S	S					N	P	Cured
71	2	Female	Term	Assisted	3000	N														N	N	Cured
72	2	Female	Term	LSCS	2900	N														P	P	Death
73	4	Male	Preterm	Normal	2100	P	E.coli	S	S	S	S	S	S	S	S					N	P	Cured
74	4	Male	Preterm	Normal	2200	N														N	N	Cured
75	17	Female	Preterm	Normal	2000	N														N	P	Cured
76	4	Female	Term	LSCS	2100	N														N	N	Cured
77	10	Male	Preterm	Normal	2400	N														N	N	Cured
78	3	Male	Term	LSCS	2300	N														P	P	Cured
79	2	Female	Term	Normal	2700	N														N	N	Cured
80	3	Female	Term	Normal	2800	N														N	N	Cured
81	5	Male	Preterm	LSCS	2400	N														N	N	Cured
82	2	Male	Preterm	Normal	2000	P	K.pneumoniae	S	S	S	S	S	S	S	S					N	P	Cured
83	3	Male	Term	LSCS	2900	N														P	P	Cured
84	1	Male	Term	Normal	3100	N														N	N	Cured
85	1	Female	Term	Normal	2500	N														N	N	Cured
86	2	Female	Term	LSCS	2400	P	E.coli	S	S	S	S	S	S	S	S					P	P	Cured
87	2	Female	Preterm	Normal	1900	N														N	N	Cured

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88	4	Male	Preterm	Normal	2100	N														N	N	Cured
89	3	Male	Term	LSCS	2600	N														P	N	Cured
90	3	Female	Preterm	Normal	2200	P	P.aeruginosa	S	S	S	S	S	R	S	S					N	P	Cured
91	4	Male	Preterm	Normal	2000	P	E.coli	S	S	S	S	S	R	S	S					P	P	Cured
92	5	Female	Preterm	LSCS	2200	P	K.oxytoca	S	S	R	R	S	R	S	S					N	P	Cured
93	4	Male	Preterm	Normal	1900	P	K.oxytoca	R	R	R	R	S	R	R	S					N	P	Cured
94	1	Female	Term	LSCS	2900	N														P	N	Cured
95	2	Female	Term	LSCS	3000	N														N	N	Cured
96	6	Male	Preterm	Normal	2200	N														N	N	Cured
97	4	Male	Preterm	Normal	2100	N														N	P	Cured
98	3	Male	Term	Normal	3100	N														N	N	Cured
99	4	Male	Preterm	Normal	2400	N														N	N	Cured
100	2	Female	Term	LSCS	2600	N														N	N	Cured